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ORIGINAL ARTICLES

IMMUNITY IN SYPHILIS*

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I.

UNTIL relatively recent years there seems to have been little question in the mind of clinicians regarding the existence of true immunity in syphilis. It was stated uncompromisingly by Ricord that "an individual who had once acquired syphilis was thereafter protected against reinfection." This opinion acquired wide acceptance and was shared by most of his contemporaries. Bäumler in 1875 summarizing the authoritative opinions of that period stated that "One who has once had smallpox, scarlet fever, typhus, etc., is, as a rule, not liable to these diseases again for the rest of his life. The same is true of syphilis." However, even at the time Bäumler wrote this, exceptions to the supposed rule were accumulating and he appends, to the positive statement given above, references to observed instances of second infection reported by Bidenkap, H. Lee, Diday, Köber, Zeissl, and others.

The conception of the existence of a true acquired immunity was also expressed and widely accepted in the so-called "laws" of Colles and Profeta. The former, first enunciated by Beaumès and later, in 1837, stated by Abraham Colles, of Dublin, is the well-known generalization based on the observation that mothers who have borne syphilitic infants were not infected by their children while suckling them, although such children might often infect wet nurses. Profeta's observation was the converse of this, namely, that children born of mothers who suffered from active syphilis during the period of conception did not acquire the disease from their mothers.

Both of these phenomena appear too well founded in clinical observation to be questioned as, at least frequent occurrences. Moreover, considering the intimate contact between mother and infant during the first months after birth, they acquire unusual importance. However, as we shall see, they have been deprived of much of their bearing as proofs of true acquired or inherited

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immunity by serological investigations such as those of Bauer, Knöpfelmacher, and others, which have shown that mothers of syphilitic children usually give positive Wassermann reactions, a fact which makes it seem likely that such women are suffering from syphilis in a latent form and are not immune in the ordinary sense. This indeed was the interpretation given to the "laws" of Colles and Profeta by Fournier and by Matzenauer at a time prior to that at which serological data were available. It is still unclear why such mothers should so frequently exhibit the disease in a latent form. However, this is a matter for the intelligent discussion of which we are not at the present time in the possession of sufficient information.

In the years just preceding the period of experimental investigation of syphilis upon animals much purely clinical research was carried out on the problem of reinoculation and what is commonly known as "superinfection" of syphilitic human beings. Much of this work is unavailable as scientific evidence owing to the difficulties of distinguishing, at that time, between the true chancre and the chancroid, but a considerable number of the observations then made have been of much value in pointing out directions of later research upon animals. The work has been so thoroughly analyzed both by Neisser and by Levaditi that it would be needless repetition to do so again. The records include both accidentally occurring superinfections, and purposeful experimental reinoculations. Without, therefore, going into details concerning the individual cases we may summarize the conclusions justified from a study of these observations.

1. The reports of Lasch, Jadassohn, Sabearéanu, Queyrat, Taylor, H. Lee, Knowles, and many others, have shown that patients are susceptible to a second inoculation during the first incubation time, that is, during the period elapsing between the first infection with syphilis and the appearance of the chancre. Second positive inoculations have also been successful at periods shortly subsequent to the appearance of the primary sore.

Autoinoculations and reinoculations undertaken after the chancre has become well developed have been, in the main, negative, though Queyrat reports a case successfully inoculated daily up to the eleventh, and Taylor one inoculated on the fourteenth day after the appearance of the primary induration. In contrast to these and other successful attempts, many observers record failure. However, the point is not one of particular importance, since, after all, the appearance of the chancre does not mark off any fundamental change in the progressve pathological development of the disease, and indicates only the completed reaction at the point of entrance. The fact remains that analysis has revealed that reinoculation, with the appearance of the second initial lesion, is possible up to about the twentieth to the thirtieth day after the first infection with the treponemata (Mauriac and Neisser state the twenty-second day, Queyrat cites a case eleven days, Linderman one twenty-four days after the appearance of the chancre). It is claimed by some of the observers, however, that even when reinoculation during this period is successful, the incubation of the second and subsequent lesions is shorter, that the induration itself is less severe, may not ulcerate, and heals more readily than the first.

In judging of the success or failure of reinoculation practiced during the

later days of the period above referred to, the possibility must be borne in mind that the trauma produced at the inoculation might have served to favor the development of a localized focus, the treponemata at this time being very probably well distributed throughout the body. Unsuccessful control inoculations with non-syphilitic materials in Queyrat's cases would tend to eliminate this possibility, whereas lesions resulting at the sites of such control abrasions in the experiments of Neuman and Cehak, and of Levaditi, appear to support it. However, this is not of great importance inasmuch as it determines merely a relative lengthening or shortening of the period during which reinoculation or superinfection is possible.

2. After the disease is well established as a systemic infection, that is, from the time of development of the chancre throughout the so-called active "secondary" period, reinoculation is either impossible or, at any rate, extremely difficult. Neisser cites Rollet as follows:

"Although I and my predecessors have a thousand times attempted to reinoculate luetic subjects, we have never observed a successful case. I know no single fact more thoroughly proven than the insusceptibility of a syphilitic to the action of a new virus, and, moreover, these experiments are so harmless that they may be performed without scruple."

The same opinion was held by Mauriac and is in a general way assented to by Neisser in his summary of this place of his studies. (Neisser, loc. cit. pp. 180-181.)

That the patient with well-developed lues has acquired a considerable degree of resistance to fresh inoculations is pretty generally accepted, therefore, by all who have studied the question—but there are investigators, notably Finger and Landsteiner, who believe this resistance to be less absolute than stated by earlier writers. Their observations on patients with active syphilis seem to indicate that superinfection is possible "under certain circumstances in all stages of the disease," but "the positive effect can be obtained only with considerable quantities of the virus." Furthermore, Landsteiner states that, in general, lesions so obtained are relatively slight in severity, do not appear as primary indurations, but have the tendency to simulate the particular variety of lesion spontaneously manifest in the individual at the time. Were it not for Finger and Landsteiner's monkey experiments, to which we will refer directly, and which seem to bear them out in their interpretation of these inoculations as "positive" results, the simulation of the spontaneously occurring lesions by the inoculation-products would again justify suspicion that these experimental results represented merely traumata in which, as points of less resistance, the patient's pre-existent disease had found a favorable spot for localization.

Observations in this respect, also, are corroborated by the older literature. A report which has direct bearing on this point, is one of Queyrat and Pinard who inoculated a tertiary patient with chancre material, obtaining not a primary sore but an ulcerated lesion having the clinical characteristics typical of the late skin manifestations of the disease. The autoinoculation experiments of Ehrmann also would tend to show that the resistance of the luetic subject is a relative one only. Ehrmann succeeded in producing positive autoinoculation-products in 45 syphilitics with papular eruptions. Control inoculations with

sterile water were negative. Although his observations and those of Finger and Landsteiner, with other similar ones, teach us that superinfection during the disease is possible the nature of the lesions, their short incubation time, and their exceptional character when averaged with the total of such attempts, prevent them from invalidating the conclusion that there is a resistance at this stage higher than that of the normal subject.

We may conclude, therefore, concerning the secondary period of the disease, that the luetic individual has acquired a resistance which while not absolute is at least very high, and protects him from fresh external inoculation, although at the time his disease may still be progressing and in no sense overcome.

3. During the late stage of syphilis, the stage at which, according to the more or less arbitrary divisions of Ricord, we are accustomed to speak of it as "tertiary," the resistance is still manifest, though apparently not so regularly potent as during the preceding "secondary" period. Neisser expresses himself with great caution and accepts few, if any, of the observed reinoculations of tertiary cases as surely representing unquestionable freshly acquired infections. Nevertheless, taking into consideration a detailed study of individual reports, he concludes that resistance during the late stages is pronounced but already beginning to wane. He himself in the Festschrifft to F. Joseph Pick in 1898 cites a case of the development of a chancre in a tertiary case which, however, was not followed by constitutional symptoms.

4. In the preceding paragraphs we have concerned ourselves entirely with questions of "superinfection," that is, the implantation of the syphilitic virus into subjects still suffering from manifestations of the disease. A problem of equal theoretical, and of much greater practical importance, is that dealing with true "reinfection." By "immunity" in the ordinary sense, we mean an increased resistance to specific infection which persists for a more or less prolonged period after the active disease has been overcome and the causative agents removed from the body. It is not easy to draw conclusions concerning this point from observations on human beings, since it is most difficult to decide, even with the aid of serological methods, whether or not a given case is cured in the bacteriological sense; for syphilis is pre-eminently the disease in which there occur frequent and prolonged latent periods terminated, often after lapses of years, by the reappearance of foci of a grave nature.

Moreover, our own experiments both with syphilis in rabbits and the treponemata in culture have convinced us that these microorganisms may assume for long periods a condition of metabolic latency, a sort of resting period, during which they incite no reactions of any kind on the part of the tissues, apparently do not multiply to any great extent and yet remain alive and capable of development when conditions favor this.

In spite of these difficulties, however, careful clinical studies by Jonathan Hutchinson, Taylor, Hudélo, Neisser, and many others, have furnished data which warrant an opinion upon this problem. Of especial value is the painstaking analysis of reported cases of reinfection in syphilis made in 1909 by Felix John.

In contrast with some others who have attempted similar analyses John

takes the utmost care to separate these cases into the ones in which the evidence of true reinfection is absolute, and those in which the reported details are insufficient to exclude the possibility of recrudescence. In agreement with Taylor he insists upon a symptom-free interval of five years between the last manifestations of the first attack and the appearance of the second. As an example of what he calls an "Ideal Fall" we may cite the following:

X. J., April 1, 1872, primary sore.
Roseola, polyadenitis, mucous patches. September, 1872, papular rash. March, 1873, palmar and plantar spots, iritis. 1875, gumma of tibia and sepriginous syphilide of right thigh.
Four courses of inunctions and KI.
1876, married—two healthy children.
No symptoms till 1887 when he acquired a second chancre followed by typical roseola. In 1888 wife had still-birth.

John has analyzed in this way 356 cases of supposed reinfection, in 34 of which the first attack was of congenital origin. Of the remaining 322, fourteen were cases which seemed unquestionable instances of reinfection and in 16 more there was practically no doubt of this. Of the 34 hereditary cases there were three, one of Emery, one of Taylor, and one of Hochsinger, in which there was practically no question of their nature as valid reinfections. In all of the others there was one point or another which rendered them doubtful as evidence.

John concludes that true reinfection can unquestionably occur in syphilis but that it is relatively rare.

To John's cases Neisser has added others reported between 1909 and 1911. Yet even with these, the total number is not a large one. Nevertheless, we should not be tempted to conclude from this that the relative infrequency of such cases is evidence in favor of the existence of a true immunity analogous to that following typhoid, plague, etc. For we have seen that a very definite insusceptibility is coincident with the persistence of actual disease in the subject and, as Neisser points out, the more recent investigations carried out with the aid of serological tests have shown that the number of cases uncured though long without symptoms, is much larger than formerly supposed. The scarcity of true reinfection, therefore, may well be due to the relative scarcity of completely cured cases. Moreover, it must be remembered that, in this disease, even when final recovery results, it is usually achieved only at an age when the individual is less exposed to reinfection because of changed economic and domestic conditions, or by reason of the virtue which comes with arteriosclerosis and the wisdom of the burnt child that fears the fire.

Granted, then, that true reinfection is possible, is there any evidence that when it does occur, the second attack is less severe and more easily cured than the first, a fact which would also tend to support the opinion that a certain degree of immunity persists. Jonathan Hutchinson expressed this view in a clinical lecture in which he states that "second chancres are far more common than second attacks of constitutional syphilis." However, John in his summary, in which Hutchinson's cases are included, finds that in general the disease has run a second course very similar as to severity to that incident to the first infection.

If we gather together, then, the facts revealed by clinical study we may conclude with Levaditi, Neisser, and most others, that:

1. The syphilitic subject acquires definite resistance to reinoculation which becomes manifest soon after the appearance of the primary sore, at a time when the virus may be regarded as having gained universal systemic distribution.

2. This resistance, high though not absolute, persists throughout the secondary or most active period of the disease and into the tertiary stage. During the latter, however, it appears somewhat to decline, reinoculation or superinfection being more frequently possible at this period.

3. When syphilis is entirely cured, susceptibility may in all probability be regarded as returning, possibly, though not certainly, to the same degree as it exists in the normal subject. The reasons for this last belief will become more clear when we study the evidence contributed by animal experimentation.

II.

Notwithstanding the admirable thoroughness with which clinical data had been collected and analyzed in the study of syphilis, progress beyond the points indicated in the preceding sections was quite impossible without the aid of animal experimentation and a knowledge of the causative agent. Fortunately these two deficiencies in our methods were removed when in 1903 Metchnikoff and Roux succeeded in transmitting the disease to a chimpanzee, and in March, 1905, Schaudinn discovered the treponema pallidum.

As a matter of fact, probable transmission of syphilis to lower monkeys had been accomplished as early as 1879 by Klebs and subsequently by Neumann, Martineau, and Charles Nicolle, but in none of these experiments had it been possible to prove beyond question the syphilitic nature of the inoculation-products. In the chimpanzees inoculated by Metchnikoff and Roux, the animals developed not only primary sores but also secondary eruptions, polyadenitis, and enlarged spleens in such characteristic manner that the identity of the inoculation disease with human syphilis could no longer be doubted.

Successful transmission to other anthropoids and to lower monkeys were then announced, in rapid succession, by Metchnikoff and Roux, Ch. Nicolle, Neisser, Baermann and Halberstaedter, Finger and Landsteiner, Hoffman, and others. The susceptibility of monkeys was tabulated by Neisser in the following series: Chimpanzee, Gibbon, Orang-Outang, Cynocephalus babuin, Cynocephalus sphinx, Cynocephalus hamadryas, Cercopithecus, fulginosus, Macacus niger, M. nemestrinus, M. cynomolgus, M. sinicus, M. speciosus, M. rhesus. In 1906 Bertarelli produced syphilitic keratitis in rabbits, demonstrating the treponema pallidum in sections of the cornea and in 1907 Parodi first produced syphilitic orchitis in the same animals.

Apart from monkeys and rabbits, no animal species have so far been shown sufficiently susceptible to be available for systematic study. It is true that the production of keratitis is claimed in dogs and sheep (Bertarelli, Hoffman and Brünig), in guinea pigs (Bertarelli), in cats (Levaditi and Yamanouchi), and in goats (Bertarelli). However, these experiments have been isolated and too uncertain with present methods to offer material for experimentation. Our own attempts on cats, pigs, guinea pigs, rats, mice, and a few birds, have yielded

negative results only. There are many observations of great scientific interest which might be discussed in connection with the problem of susceptibility of various animal species, however, we will confine ourselves at present to those phases of the work only which have bearing on the questions of immunity.

In the fundamental premises, the work on monkeys has pretty accurately confirmed the observations concerning reinoculation and superinfection previously made on human beings.

The most extensive studies in this respect are those of Neisser and his associates in the Javan expedition. Briefly stated, Neisser reinoculated 135 animals 165 times with negative result on the second and subsequent inoculations. The second inoculations were made at periods ranging from 21 days to two years after the first. In but 27 animals did the reinoculation show positive results, and in ten of these cases only does Neisser recognize the experiments as valid. Although these ten positive reinoculations it is true, add an element of irregularity to the series, they constitute but 6.8 per cent of the entire number, a proportion which in no way invalidates the experiments when we consider that the work was done entirely on the lower monkeys, animals that are far less susceptible to syphilis than are human beings and in many of which, therefore, systemic distribution of the virus (a generalization apparently necessary for the development of resistance) may not have taken place. Neisser's conclusions, therefore, that monkeys, like human beings, are not reinoculable while suffering from systemic syphilis, seem entirely justified.

His work, as well as that of Finger and Landsteiner, and of Kraus and Volk on lower monkeys, has shown that resistance does not develop until the twelfth to the twentieth or twenty-first day after the first inoculation; that is, again as in man, when the virus has become generally distributed. Finger and Landsteiner, furthermore, noticed that reinoculation-products, obtained by reinfection during the first incubation period; that is, before the development of the primary lesion, were less severe and developed in a shorter time than did the first lesion. This phenomenon which would tend to mark another analogy to the conditions prevailing in human beings, was not observed in the experiments of Neisser and of Kraus and Volk. However, like the similar observation in human beings, it seems to indicate the gradual acquisition of resistance as the virus begins to exert its influence upon the tissues.

Again, with monkeys as in man, the question arises whether the resistance so unquestionably proven is a condition merely coexistent with active disease, or whether it may be interpreted as a true immunity which persists after the microorganisms have been completely removed. The most directly pertinent experiments are those of Neisser. Neisser reinoculated monkeys at periods ranging from 27 to 645 days after the first infection. After waiting a time sufficiently long to insure the negative result of the reinoculation, he used organ-substance from these animals to inoculate other monkeys. In 22 experiments of this kind he obtained positive results—showing that the organs of the apparently immune animals still harbored virulent treponemata. In seven animals only did the inoculations with organ-substance fail to produce lesions, but of these all but one died before the 30th day after inoculation.

In contrast to these results Neisser found that animals which had been

"cured" by various treponemacidal agents such as Atoxyl, Arsacetin, etc., were almost regularly reinoculable. In fact, these experiments were so uniform that Neisser later utilized the reinoculation method as an index of cure or persistence of the disease.

The results obtained in monkeys, therefore, are very similar to those determined by clinical observations in man, and the following statements may be taken as summarizing the conditions revealed by monkey experiment.

1. That the body develops resistance, progressively increasing as the virus becomes generally distributed.

2. That the resistance probably reaches its highest development during the early tertiary period.

3. That complete cure is probably synonymous with gradual return of susceptibility.

The only other animals on which systematic experimentation has been possible up to the present time, have been rabbits. Since Bertarelli's successful production of keratitis and Parodi's inoculation of the testes in these animals, they have been studied carefully by a number of workers, chiefly Uhlenhuth and Mulzer, and Noguchi; and in our own laboratory, with Hopkins and McBurney, the writer has observed rabbit syphilis for a number of years. From a large mass of observations it appears that the conditions in rabbits are not identical with those observed in man and monkeys. So far, the testes and the eyes are the only organs in these animals in which syphilitic lesions can regularly be produced, and although treponema pallidum may apparently be distributed generally to the organs after inoculation, it does not easily arouse pathological reactions except in the organs named, and the lesions produced are not accompanied by a generalized resistance comparable to that discussed in connection with the higher animals in preceding sections of this paper.

Bertarelli found that he could reinoculate the cornea in a rabbit that had previously been inoculated with syphilis. Uhlenhuth and Mulzer, and Neisser and Pürckhauer, showed that infections of the eye could be produced while the opposite eye was still syphilitic. Tomazewski found that scrotal infection did not protect against infection of the cornea, and vice versa. Furthermore, Uhlenhuth and Mulzer, on the basis of a very thorough study, believe that such reinfections are neither less extensive nor more rapid in healing than was the first lesion. The same authors noticed resistance to reinoculation in two animals only, and these were young rabbits in the first weeks of life, which, they believed, had been generally syphilized by intracardial injections. Interesting in this connection are claims by Ossola and Truffi who believe that successful skin inoculation in rabbits confers a certain amount of skin resistance and this is in harmony with the belief of Kraus and Volk, that a specific skin immunity in syphilis is possible. Of great interest to us and of a possible theoretical bearing which we will discuss below, are observations made by the writer with Hopkins and McBurney on 20 rabbits which were reinoculated into the testes after apparent healing of lesions in these organs. It appeared that in rabbits the opposite testis can be successfully inoculated before, during, or after, the existence of a testicular lesion on one side, but that reinoculation of the same

testis which had apparently returned to normal, at periods ranging from 6 weeks to one year was not often successful. There is a certain amount of evidence here of a purely local immunity, a matter which we will discuss at greater length presently. Perhaps the difference between rabbits and the higher animals lies chiefly in the fact that syphilis is not generalized in the same sense that it is in man and monkeys, and even though inoculations from the organs of syphilitic rabbits may often result positively, this might signify only that the treponemata have been generally distributed by the blood stream and latently lodged in the organs, without, however, arousing in the tissues any sort of pathological response. This idea would seem to be borne out by the two experiments of Uhlenhuth and Mulzer cited above in very young rabbits since in such animals true generalization seems to be more common. The study of very young rabbits will be continued with this point in view.

It is apparent from the preceding considerations that resistance to syphilis differs from that acquired in many bacterial infections chiefly in the fact that it does not persist after the disease is over, but probably coexists only with the presence of the living incitants in the body. In this respect it seems to be similar to the conditions prevailing in many protozoan diseases. Thus Schilling cites a case of Trypanosoma Brucei in a steer, experimentally infected by Koch, in which reinoculation was repeatedly negative, this result being at first falsely interpreted as immunity; but six years later Kleine found that the same steer still harbored the trypanosomes in his blood—showing that the resistance to reinoculation was not, in this case, an evidence of true immunity, but rather represented a condition of insusceptibility to "superinfection" analogous in every respect to that existing in syphilis. Similar conditions have been shown to prevail in Texas fever and Schilling believes that they may be regarded as also existing to a certain extent in Malaria and Sleeping Sickness. In both of these conditions complete spontaneous sterilization of the body (without medicinal aid) is probably rare, possibly does not occur at all, and the apparent immunity to reinfection is, as in syphilis, an evidence of persistence of the disease in a latent form.

In weighing the analogy of syphilis to such protozoan diseases, one is inclined to wonder whether syphilis in man may be regarded as at all spontaneously curable. So few are the cases left untreated and so rarely does the reinfection occur even in the face of specific remedies, that it seems to us more than likely that in syphilis, as in Sleeping Sickness, a spontaneous "sterilizing" immunity does not occur. This is a point, however, regarding which it is impossible to gather data.

In order to distinguish the conditions outlined above tersely from the ordinary conception of immunity, Neisser speaks of the alterations which govern the reactions of the luetic body to freshly introduced virus as "Anergie;" and "Umstimmung" or "*Allegerie.*" By "*Anergie*" (a term first used by v. Pirquet and subsequently introduced by Siebert in working out the analogy between pigeon-epithelioma and syphilis), Neisser designates a condition of inability to react by cellular change to contact with the virus. As he uses it, it implies a passivity on the part of the invaded tissues (in which "die Zellen auf die Spirochaeten schwer oder garnicht reagiren), by which there is not necessarily a destruction

of the invading treponemata, and which, therefore, cannot in any sense be interpreted as a "Schutz wirkung." By the "Umstimmung" of Neisser, the "changement dans la mode de reaction" of Levaditi, is meant the changed reaction capacity of the syphilized tissues which determines the characters of the lesions at various stages of the disease. Thus it is obvious that cellular reactions which result in the primary induration are quite different from those which produce the tertiary gumma, and that the histological changes of the roseola are distinct from those of the serpiginous syphilide of the late stages. And since, as we shall see, there is no valid reason to assume that the incitant has been modified in virulence or vitality, we are forced to believe that the reaction capacity of the body cells has been altered.

III.

Since it is a fact, then, that syphilitic infection so changes the body tissues of man and monkeys that, during its course, resistance to reinfection is produced, it should be possible to analyze this resistance into its responsible factors and perhaps utilize the knowledge so gained for practical therapeutic purposes. Before we proceed to do this, it will be of advantage to review briefly the attempts at active and passive immunization which have been made in animals and man.

Metchnikoff and Roux followed their first animal inoculation studies by extensive vaccination experiments. Some of their first work along these lines is perhaps marred to some extent by an insufficient recognition of the resistance which depends upon persistence of the disease rather than upon true immunity, but a good many of their observations are of fundamental importance. It will be convenient to classify their work and that of others into experiments dealing with "active" and those dealing with "passive" immunization.

Metchnikoff and Roux first worked with filtered virus and virus killed at 51° C. All their attempts with such material were negative in that the monkeys treated with it could not be regarded in any sense as immunized. In their reports of these experiments they remark that they believed this to be due to an absolute loss of power to incite reaction on the part of the vaccine-material. We emphasize this point here because our own subsequent work inclines us to believe, with them, that the production of a reaction is necessary for the development of any considerable degree of resistance.

Neisser carried out a large number of attempts at vaccination in which he used extracts of syphilitic primary lesions and of the organs of congenitally syphilitic children, killed by the addition of carbolic acid. Unfortunately he assumed that his extract contained syphilitic antigen because it gave positive reactions by the complement fixation technique of Wassermann, an assumption which we of course know now to be unfounded as far as any relation to the body substance of the treponemata is concerned. This, to our mind, deprives these particular experiments entirely of their negative importance.

In rabbits a large amount of work has been done by Uhlenhuth and Mulzer. They injected living material from rabbit lesions intravenously and subcutaneously, without ever observing any evidence of protection against subsequent inoculations.

Of perhaps the greatest importance in connection with active immunization are the attempts made upon human beings by different investigators.

Casagrandi and de Luca tried prophylactic immunization on six human beings by injection of filtrates obtained from primary lesions. Two of these people later contracted syphilis in the ordinary way.

Possibly the most hopeful results are those obtained by Spitzer by a method suggested by Kraus. Kraus, reasoning from the fact that syphilis like hydrophobia was a disease with long incubation time, expressed the hope that perhaps the method of Pasteur in hydrophobia, that is, active immunization during the period of incubation might, in syphilis also, tend to abort the disease. Accordingly, Spitzer treated 15 cases of early syphilis immediately after the appearance of the chancre by subcutaneous injections of emulsions made from human chancre material in dilutions of 1:200 to 1:20. The cases received from 11 to 20 injections and in seven of them the disease was uninfluenced. In the others, however, subsequent symptoms were delayed, and in four, no generalized symptoms occurred. In a later communication, Spitzer reported 23 further cases similarly treated, 10 of which failed to develop generalized symptoms and in 9 of these the Wassermann test remained negative. One of them, a fact which is of great importance, was spontaneously reinfected with syphilis two and one-half years later. These results if accurate in every way, are of the greatest importance, but are diametrically opposed to the experience of all other investigators. Monkey experiments along the same lines by Neisser gave entirely negative results and Brandweiner as well as Kreibich were unable to confirm Spitzer's results in man. Further comment on the Kraus and Spitzer method is valueless without more experimental data. It is one of the few rays of hope but so isolated that one is forced to skepticism. Metchnikoff and Roux did almost the identical thing in an orang-outang and obtained lesions both at the point of the original inoculation as well as that at which the subsequent "protective" injection was made.

The only experiments in which an attempt at vaccination with *attenuated virus* was made with some indication of efficacy, is one of Metchnikoff and Roux, the outcome of an accidental laboratory infection. It appears that a laboratory assistant who had been attending to the animals, noticed a small lesion on his lip which did not look like a typical syphilitic chancre. In order to allay the patient's fears, however, Metchnikoff and Roux did inoculations from the patient to monkeys and these were positive. Nevertheless, Fournier after examining the original lesion declared it so unlike the ordinary primary sore that he did not advise treatment. No secondaries developed in the patient nor in the three chimpanzees inoculated with the material. From this occurrence Metchnikoff and Roux concluded that the patient had probably been infected in handling the monkeys, and that the virus had become attenuated by passage through these animals. On the basis of this observation they later inoculated a willing subject 79 years old with virus carried for five generations in lower monkeys. The lesion which developed was very slight, consisting only of a local induration and no generalized symptoms developed. A previous attack of syphilis Metchnikoff believes could be reliably excluded in the subject. The experimenters suggest that the passage through lower monkeys may attenuate the virus for man, this leading to relative immunity to subsequent inoculations, and furnish a possible means of protection. Their experiments are too few to

permit conclusions as yet, but even should they hold good, the method would seem to imply a considerable degree of risk and our experience with superinfections and reinfections in syphilis does not encourage the hope that a method so drastic would be justified when the benefit to the patient is apt to be of such duration only.

The history of passive immunization is an extensive one and hardly worth going into with any degree of detail since many times extravagant claims have been made only to be refuted by accurate study. There is a great similarity in respect to this between syphilis studies and those in tuberculosis and cancer. A brief examination of the bibliography in Neisser's book is sufficient to convince one of the many attempts that have been made in this direction and often by methods as ludicrous as the claims of success for which they formed the basis. The most careful and skillful workers have uniformly reported failure. Metchnikoff and Roux treated various monkeys with blood from syphilitic patients and used the serum of these animals for protective experiments. There are a few instances in which mixtures of such serum with syphilitic virus rendered this inactive on inoculation. A powder made of this serum was supposed to have some protective effect when applied to fresh inoculation spots within the first hour after inoculation. However, injection of the serum had no effect whatever. Casagrandi and de Luca using serum of a dog treated with syphilitic virus, obtained entirely negative results, and Finger and Landsteiner report negative results with monkey blood in man. The most extensive experiments were again those carried out by Neisser and his associates. They were done by the treatment of animals with dead and living syphilis virus, organ extracts, with the blood of syphilitic man and monkey, and horses, sheep, and monkeys were used for the production of "immune" serum. In no case was there the slightest protective effect on the part of the serum either *in vitro* or *in vivo*, and the results of the experiment were unqualifiedly negative.

IV.

When the method of complement fixation was successfully applied to the diagnosis of syphilis first by Detre, and then by Wassermann, Neisser and Bruck, it was generally assumed that this reaction incidentally demonstrated that specific antibodies were formed in syphilis. As matters have developed, however, this point of view can no longer be maintained. It was soon discovered that the antigen used for these reactions by Wassermann and his associates derived its "fixing" constituent not from the body substances of the treponemata contained in the syphilitic organs, but from certain tissue extractives chiefly of lipoidal nature which could be obtained readily from normal as well as syphilitic tissues. Although Bruck and others who have occupied themselves with the theoretical basis of the Wassermann reaction, still maintain that a specific antibody may be incidentally involved, they admit that this is the less important factor in the reaction which depends chiefly upon the existence of lipotropic substances which appear in the course of the disease as metabolic products either of the body or possibly of the treponemata. This is a subject which we will deal with in extenso in another paper. It is sufficient for our present purposes to point out that, although to a slight extent specific antibodies may play a part

in the Wassermann reaction, this is certainly not the chief element or even a very important factor involved. The truth of this conception has been further confirmed by the work of Noguchi, of Craig and Nichols, of Kolmer, and ourselves, in which it has been found that antigens made with pure cultures of treponemata produced a complement fixation with syphilitic sera to a very limited extent only, and in our work in which we have been able to duplicate the Wassermann reaction in a large series of antigens made from the treponema cultures, we have found that similar results could be obtained with cultures of colon and typhoid bacilli identically prepared, the fixing power to a large degree depending upon the lipoidal constituents of the bacteria. Whatever the ultimate explanation of the Wassermann reaction may turn out to be (and this is a subject which it would not be profitable to discuss at length at this place), it cannot be maintained that as it exists at present, it can be interpreted as demonstrating the existence of true circulating antibodies, analogous to those found in bacterial diseases in the blood of syphilitic patients.

Early in the history of such investigations, Fornet with his collaborators Schereschewsky, Eisenzimmer, and Rosenfeld, found that when the sera of syphilitics were mixed with clear extracts of syphilitic livers similar to those used in the Wassermann reactions, precipitates formed which were not seen in similar experiments done with normal sera. This Fornet in a number of communications interpreted as showing the formation of precipitins in the course of syphilis. At almost the same time, L. Michaelis made similar observations giving them the same interpretations. The experiments of Fornet have not found universal confirmation, but even if such precipitin reactions can be occasionally observed, they do not indicate true precipitins for the same reasons that the Wassermann reaction does not demonstrate true complement fixation of antibodies. The antigens did not represent strong treponema antigens and Jacobsthal and others have shown that with the ordinary Wassermann antigens syphilitic sera can produce precipitations visible under the dark field, the basis of the complement fixation being, therefore, one of probable colloidal precipitation. The formation of true precipitins, therefore, has not been shown for syphilis.

Investigations of the effect produced upon virulent treponemata by the sera of syphilitic individuals have likewise been unsatisfactory. Hoffman and Prowazek reported in 1906 that the serum of syphilitics in the later stages produced immobilization of the virulent treponema pallidum, an observation which was confirmed by Zabolotny. Landsteiner and Mucha were not able to observe such immobilization, nor did they see any evidence of agglutination in such experiments. In a limited observation of our own, we have also failed to see any regular or distinct influences of this kind. It is not impossible that a slight difference may exist in this respect between syphilitic and normal sera, but even if it occurs, the action is feeble, irregular, and entirely insufficient to be interpreted as having much practical importance in the acquisition of syphilis immunity.

Treponemacidal experiments have been done by some workers also with negative results. As we have stated in another part of this paper, the attempt to treat syphilitic animals and man with the serum of syphilitics has led to no

reliable results, and no evidence whatever that can be accepted has been adduced to show that virulent treponemata may be killed by active luetic serum.

As far as any opsonic action is concerned, Levaditi in histological studies in congenitally syphilitic children has seen phagocytosis or at least intracellular localization of treponemata in the alveolar cells of the lung and in the parenchyma cells of the liver and kidneys. For reasons not entirely clear to us, he interprets the former as true phagocytosis and the latter as a penetration of the treponemata into the liver cells to the detriment of the latter. We ourselves have occasionally seen phagocytosis in sections of syphilitic rabbit testes, but in all cases the process was not a very active one and not much can be said about its importance at present. As a matter of fact, Hopkins and the writer, in studying the mechanism of the natural resistance of mice against syphilis, injected virulent organisms into the peritoneal cavities and observed the treponemata in peritoneal puncture fluid, alive, actively motile, and unphagocyted, though surrounded by masses of leucocytes, as long as three days after their injection. It seemed almost as though the natural immunity of such animals might be similar to the "atreptic" cancer resistance spoken of by Ehrlich. The treponemata did not multiply in the mice but though, naturally, diminishing in number, were apparently neither killed nor even inhibited in motility by the peritoneal exudates and, for several days, swam in and out among the accumulating leucocytes, often adhering to them peripherally but not taken up by them. Lack of entirely satisfactory methods of staining cells containing treponemata make it difficult to speak with certainty of the actual occurrences. But we gained the distinct impression that the treponemata were not actively injured or destroyed until they had spontaneously died out owing to lack of suitable environment, i.e., nutrition.

In the case of natural immunity at any rate, we do not think that phagocytosis by the mobile leucocytes plays a primarily important role. However, these experiments will need further elaboration.

The search for antibodies gained new vigor when the efforts of Schereschewsky, Mühlens, Hoffman, and especially Noguchi, had resulted in successful cultivation of treponemata from syphilitic lesions in man and animals. It was hoped that with the causative organisms isolated, immunization and a clear understanding of the antibodies in syphilis might yield practical results. Kolmer observed that cultivated treponemata were agglutinated in the sera of rabbits treated with culture material, and such agglutinins were produced in high potency by the writer with Hopkins. Subsequently in our own laboratory with Hopkins and McBurney, extensive experimentation on the production of antibodies with culture pallida were carried out.

We were able to show that not only were agglutinins formed by the immunization of rabbits with such cultures, but also that treponemacidal antibodies which were analogous to the ordinary bactericidal substances in such sera were present. Also, it was shown by cross agglutination and absorption experiments, that treponemata cultivated from various sources were related in group reactions.

Indeed, experiments done with cultivated treponemata (much facilitated by the discovery of a simple method of obtaining mass cultures of old strains)

seemed at first very encouraging in that animals immunized with the cultures responded by powerful antibody formation. It was a perfectly justified hope, therefore, that antibodies produced with these "attenuated" or rather "avirulent" strains might have some action on the virulent treponemata in luetic lesions. However, subsequent work in this direction disappointed such expectations. We may briefly review this work as follows:

The serum of rabbits immunized with "culture" pallida although potent against "culture" pallida, had no effect either in agglutinating the virulent organism from rabbit lesions, nor did it exert any protective influence when the virulent organisms were subjected to its action before injection.

Conversely, the serum of syphilitic rabbits showed but a very slightly increased agglutinating power for the "culture" pallida. This increase of potency in a few experiments was definite but very slight, a few of the syphilitic animals agglutinating as highly as 1:25 and 1:50, whereas most of the normal rabbits agglutinated in 1:10 and some of them 1:25.

Although Kissmeyer has recently reported that diagnostic use might be made of the fact that sera of syphilitic individuals agglutinated the "culture" pallida, we have tried this with a considerable number of cases and found that although the sera of tertiary syphilitics will sometimes agglutinate a little more highly than will the sera of normal individuals, yet many patients suffering from non-syphilitic diseases agglutinated as highly and almost as regularly as did the syphilitics.

We have come to the conclusion, therefore, as far as our work has gone, that in the syphilitic human being there is as little agglutinin formation against the "culture" treponema as there seems to be against the virulent organisms. If the slightly greater agglutinating power found in some of the tertiary syphilitics can be considered at all, the reaction is so feeble that it is negligible from the points of view either of diagnostic value or protective importance.

Furthermore, vaccination either intravenously or locally into the testis with cultures has thus far failed to protect rabbits against subsequent inoculation with virulent material, and passive immunization with sera produced with "culture" pallida has been without effect.

From all this it appears that the "culture" treponema has immunologically no relation to the virulent organism. It has lost its virulence completely, as six and more successive inoculations into rabbit testes have sufficiently demonstrated to us.

The sera produced by many injections of dead and living culture organisms, have no effect whatever on the virulent organisms in vitro, and vaccination with it does not protect against subsequent infection.

The luetin reaction is the only method by which the relationship between the two is demonstrable at all, and there too, we have to reckon with what is generally spoken of as the non-specific increased sensitiveness of the syphilitic skin.

Were it not for the production of lesions with cultures in their early test tube generations by Hoffman, and by Noguchi in a few experiments, one would be almost in doubt as to the identity of the virulent with the culture organisms.

V.

The reactions in syphilis between the invading microorganism and the invaded subject thus differ in certain fundamental premises from those prevailing in diseases caused by most bacteria. The treponema pallidum is an organism which, unlike many bacteria, is rarely subjected to the necessity of adapting itself to extra-corporeal existence during the interval between its passage from one host to another. It practically always infects directly, being inoculated from one human being to the next and has in consequence developed a very delicate parasitism not unlike that seen in certain trypanosome diseases of rats and that which we ourselves have observed in the well-known spirochaete infections of white mice. A considerable percentage of laboratory white mice have been found to harbor actively motile spirochaetes, often in considerable numbers in the blood and peritoneal fluid without there being any objective signs of illness in the animals. It is an instance of what has been spoken of by Bail as "infection without disease," and approaches what biologists speak of as symbiosis, except that the host in this case does not benefit in any way by the invasion. Even in the case of the mice, a certain amount of gradual injury, perhaps only metabolic, by the slow removal of nutritive material, is taking place. In syphilis the mutual adaptation may perhaps be less complete, a sufficient accumulation of the invaders and especially a mechanical injury of tissue cells, of closing of tissue spaces together with a certain amount of toxic action, leading eventually to pathological changes.

The virulent treponemata apparently do not arouse true antibody formation in any marked degree. When they have been cultivated and have become accustomed to the test tube conditions they entirely lose their virulence, are easily attacked by the active constituents of animal serum, and are probably amenable to phagocytosis. When such cultures, living or dead, are injected into animals they act like other specific protein antigens and incite the formation of antibodies. However, these antibodies have no effect whatever upon the virulent organisms.

In consequence, it cannot astonish us that all efforts at passive immunization with the sera of syphilitic man and animals, or with those of animals systematically treated with dead virulent materials, have been unqualified failures.

However, this does not preclude the theoretical possibility of active immunization or vaccination with such materials since, in this case, the antigen distributed from the points of injection might act upon tissue cells throughout the body. However, with the exception of the unconfirmed reports of Spitzer, all attempts to vaccinate either with dead virulent material, or with living and dead culture material, have been disappointing. The few experiments of Metchnikoff with virus "attenuated" by passage through monkeys have indeed seemed to indicate some possibility of approaching the subject from this direction, but these isolated observations have been very logically criticized by Neisser and should not bear too much weight. The observations were made on two cases only, both of them well along in life, and the validity of the important conclusions drawn rests entirely on the always problematical fulcrum of complete exclusion of previous syphilitic infection in the two subjects. Moreover, attempts in this direction would be fraught with a considerable amount of danger and it is there-

fore questionable whether experimentation along these lines is sufficiently promising to be justified. There is certainly no attenuation for man by passage through rabbits as has been sufficiently proven by a number of accidental infections, an instance of which in a laboratory Diener has been reported by Graetz and Delbanco.

We may state, therefore, as safely summarizing our knowledge of the conditions in syphilis that the resistance which undoubtedly develops during the course of the disease is one which depends upon reaction to the living virus only, cannot so far be produced in animals by systemic treatment with dead treponemata, and does not express itself in the formation of significant amounts of circulating antibodies analogous to those observed in bacterial diseases. Moreover, it is a well-known fact that the treponemata can continue to do injury to many organs and tissues at a time when reinfection by the paths of skin and mucous membranes is no longer possible.

How, then, are we to explain this peculiar state of affairs? A clue to the problem we think is found in the 20 rabbits which Hopkins, McBurney and the writer inoculated into the testes after apparent recovery from a previous lesion. Ordinarily in rabbits, as we have stated before, no generalized resistance is developed during the disease, and the opposite testis can be successfully inoculated before, during, and after the existence of a lesion on the other side. In these rabbits it was found that testes that had apparently recovered from a previous lesion, were not subsequently as easily infected as were normal testes. It has seemed to us from this as well as from a careful study of the observations of other investigators, that resistance in syphilis was probably a matter of localized reaction. Tissues which have sustained active invasion with the living virus react and gain thereby a certain degree of resistance which expresses itself in a failure to react to subsequent inoculation. This would explain why in syphilis of the human being reinoculation is unsuccessful and reinfection of the skin and mucosæ does not occur spontaneously at a period later than the early secondary stages when the virus has become systemically distributed. It would furthermore explain why in this disease organ after organ may be pathologically involved when skin infection is no longer possible. This point of view is entirely in harmony, though perhaps from a slightly different aspect, with the skin immunity suggested by Kraus and Volk, where the resistance is attributed to the tissue as a whole rather than to a local cell group. The cells which have once reacted to the living virus no longer respond, i. e., can no longer be injured for an indefinite period after recovery. However, the factors which lend them this resistance, whatever they may be, are not distributed to the blood stream in a way analogous to that in which antibodies are mobilized in bacterial diseases, and the effect of the resistance of the local area is not distributed to remote parts of the body. It is of course likely that a certain amount of phagocytosis of treponemata by the now resistant fixed cells may account for the absence of local injury. This, however, we have not yet been able to prove sufficiently and further studies on this point are necessary. As far as any positive evidence can be adduced at the present time, the newly entering treponemata may not be entirely destroyed. It may well be that the tissues do not react and are in the state which Neisser calls "Anergie." The treponemata that enter during this period

may nevertheless remain uninjured and be as capable of subsequently causing lesions in other locations as are those already present in the patient. This phase is being studied by comparative histological observation on the fate of treponemata which have been injected into normal tissues and into tissues rendered resistant by previous infection.

In animals like monkeys and man where generalization is rapid and apparently complete, the resistance becomes a general one. In animals like the rabbit in which the lesion—or in other words pathological response, occurs in a few organs only, the resistance is limited to the particular organ or organs that have previously developed a lesion.

It must not be forgotten that such a resistance probably persists for a limited time only, and does not imply the sterilization of the body and the complete destruction of the microorganisms. These may, and probably do, remain alive and potent in various parts of the body, capable of again setting up new lesions in parts hitherto uninvolved or again susceptible after a diminution of their local, acquired resistance. That the virulent treponemata may remain thus latent, alive and virulent has been sufficiently shown in animal experimentation by successful inoculation with tertiary lesions and by the frequent late accidents, especially of the nervous system, in individuals apparently cured or for a long time without symptoms.

It would seem when we analyze the conditions in syphilis that complete sterilizing immunity or, in other words, complete cure, occurs but rarely without specific medicinal aid, and that the untreated syphilitic (if such an unfortunate individual exists in a civilized country) might go on to apparent cure, in that a general syphilization of his body would bring about a general resistance, but would always harbor virulent treponemata which could cause recrudescences in parts in which resistance was diminished, and eventually kill by degenerative processes in the central nervous system where many injuries cannot be compensated for as is possible in other organs.

The resistance which develops is apparently a new attribute only of the cell groups which have undergone direct reaction with the treponemata. This resistance may consist merely in the complete failure of the tissue cells to react to the virus, a sort of "tissue indifference" or "Anergie." It may be, however, and probably is, accompanied by a certain amount of active defense in the form of local phagocytosis of the treponemata by the fixed tissue cells.

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THE ESTIMATION OF SUGAR IN THE BLOOD BY THE LEWIS-BENEDICT METHOD. A GRAPH FOR OBTAINING DIRECTLY THE PERCENTAGE OF DEXTROSE*

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PUBLISHED less than two years ago, the method of Lewis and Benedict[1] for the determination of blood sugar has been very widely and successfully used. In this method, the red color due to the reduction of picric acid to picramic acid by dextrose, in an alkaline medium, is made the basis of a colorimetric determination. Two c.c. of blood, measured in an Ostwald pipette, are called for. Variations of the original method have been proposed by Pearce,[2] and by Myers and Bailey,[3] the same amount of blood being employed in each instance; and by Epstein,[4] who uses but a fraction of a cubic centimeter of blood.

It frequently happens, however, that it is more convenient to weigh the samples of blood than to measure them, and also that, owing to the delicacy of the method, when duplicate determinations are not essential, smaller samples are sufficient. Especially is this true when it is desirable to take a large number of samples from small animals.

The details of the method, as we have applied it, are here given.

Glass stoppered weighing bottles holding about 30 c.c. (22 by 75 mm.) are graduated to 12.5 c.c. About 3 c.c. of water are introduced into the bottle, and the whole accurately weighed. Into this about 1 c.c. of blood is allowed to drop from a small cut in the ear vein of the rabbit (as described later), care being taken to insure thorough hemolysis. The bottle is weighed at once. Seven and a half c.c. of a saturated aqueous solution of picric acid are added, the contents made up to the mark with water, and thoroughly shaken, thus precipitating completely the proteins of the blood. From this it is not difficult to obtain 8 c.c. of filtrate, if small folded filters are used, or better, hardened papers with the aid of suction. Eight c.c. of the filtrate are pipetted into a Jena glass test tube, at least 22 by 200 mm. in size, to which are added 2 c.c. of the saturated picric acid solution, 1 c.c. of a 10 per cent solution of sodium carbonate (anhydrous salt), 4 drops of kerosene (instead of mineral oil) to prevent frothing, and 2 or 3 glass beads. This solution is evaporated to dryness over a free flame. With the use of a very hot flame and constant shaking the evaporation may be accomplished in two or three minutes, without loss of material.

The residue is dissolved, with the aid of heat, in about 3 c.c. of water, transferred quantitatively to a small test tube, graduated to 10 c.c., and diluted to the mark. These test tubes have been found entirely satisfactory, and can be graduated much more easily than 10 c.c. volumetric flasks can be obtained at the present moment. Traces of kerosene that remain are removed by filtering, through small folded filters, into the colorimeter chamber or another test tube. This solution should stand not less than five nor more than twenty minutes before being compared in a Duboscq colorimeter with the standard described below.

*From the Sheffield Laboratory of Physiological Chemistry, Yale University.

Daylight from a north window is always used for reading the colorimeter, the mirror being set at the angle to give the lightest yellow tone to the standard. In testing out the method, this was found necessary in order to obtain uniform results. If it is late in the day, the material, evaporated to dryness, is left in the Jena glass tube until the next morning.

The standard color, with which the unknown solution is compared, is obtained from 0.64 mgm. of dextrose, 5 c.c. of saturated picric acid, and 1 c.c. of a 10 per cent solution of sodium carbonate, evaporated over a free flame, and

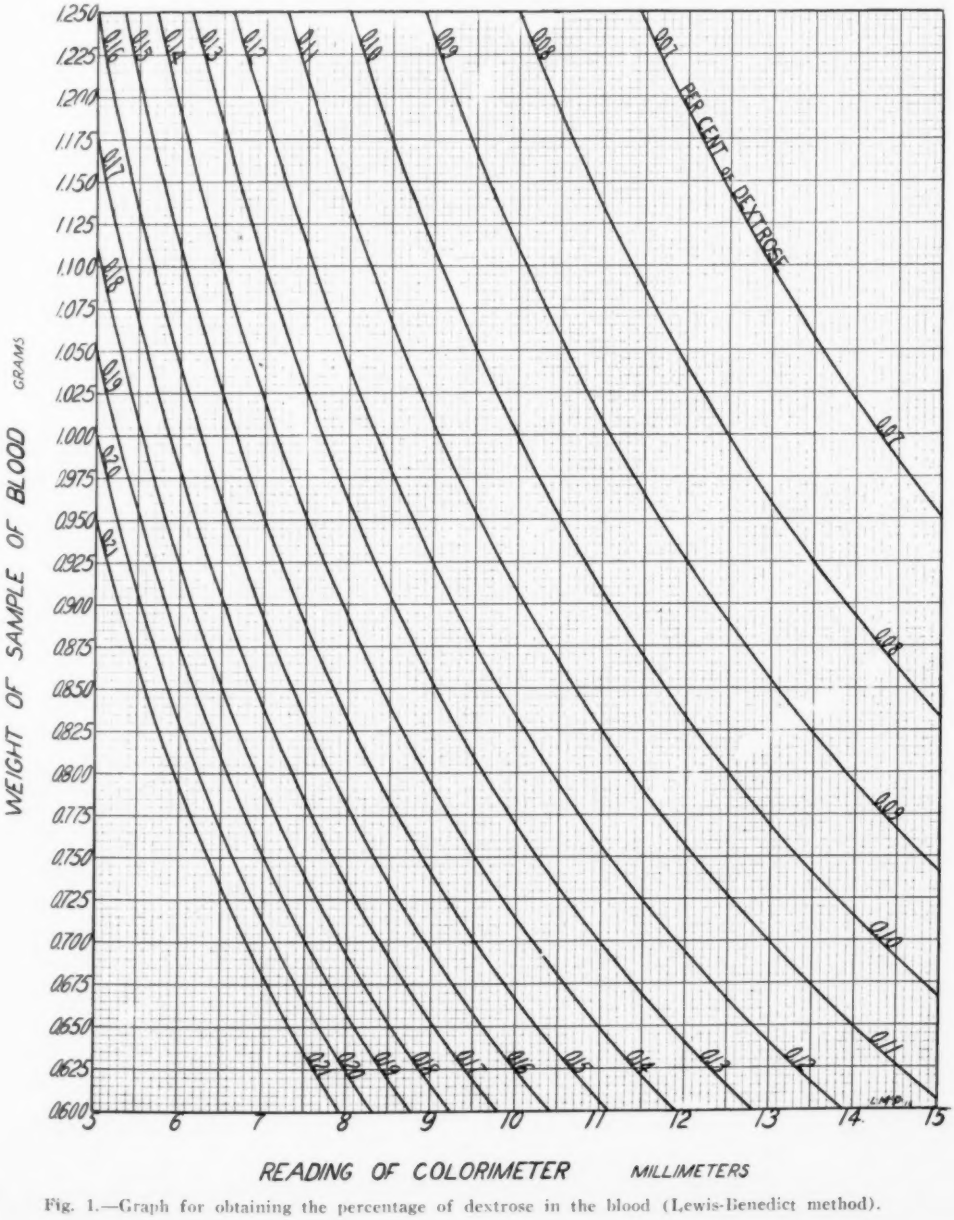

Fig. 1.—Graph for obtaining the percentage of dextrose in the blood (Lewis-Benedict method).

diluted to 10 c.c. as was the unknown. A satisfactory permanent standard consists of a solution of picramic acid, which has approximately the same color, and keeps perfectly. It requires 0.064 gm. of picramic acid and 0.100 gm. of anhydrous sodium carbonate. The picramic acid is dissolved, with the aid of heat, in 25-30 c.c. of distilled water made alkaline with the sodium carbonate. This is cooled and diluted to one liter. It should be standardized against pure dextrose.

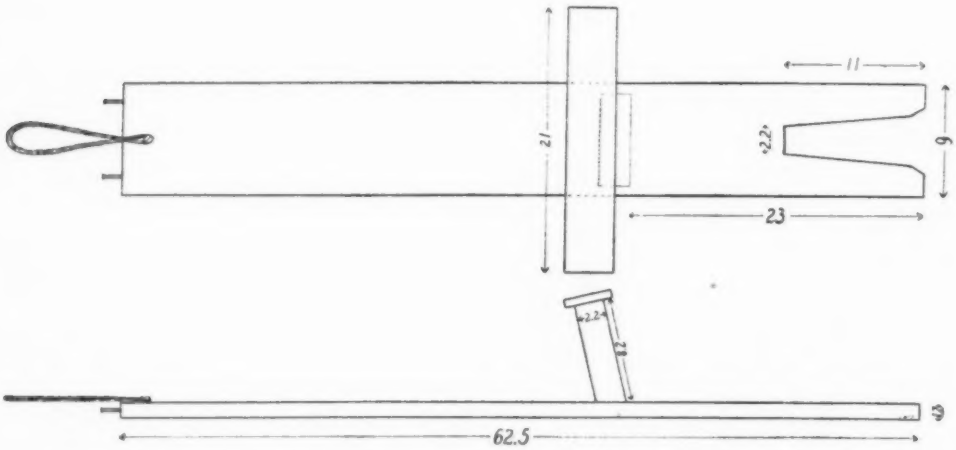

Fig. 2.—Diagram of "boot-jack" holder for rabbit. The dimensions are given in centimeters.

Fig. 3.—Rabbit fastened to board.

The general formula given by Lewis and Benedict, for the percentage of dextrose in the unknown is:

$$\text{Percentage of dextrose in blood} = \frac{\text{reading of standard}}{\text{reading of unknown}} \times \frac{\text{mgm. of dextrose in standard}}{\text{grams of blood used}} \times 0.1$$

Under the conditions here specified—the standard containing 0.64 mgm. of

dextrose, with the Duboscq colorimeter set at 10 mm., and the actual use of 8/12.5 of the sample taken—this formula becomes:

$$\text{Percentage of dextrose in blood} = \frac{1}{\text{reading of unknown} \times \text{weight of sample—grams}}$$

To avoid calculations, the accompanying graph (Fig. 1) was constructed. Weights of blood are represented by the ordinates, and colorimeter readings of the unknown by the abscissas. For a given sample, the relation of the point of intersection of these two lines to the percentage curves between which the point lies is readily determined. For example, if the sample of blood weighs 0.89 gms., and the reading of the colorimeter is 10.7 mm., the sample contains 0.105 per cent of dextrose. The points on the curves were calculated from the formula, which is used whenever either of the values lies beyond the range of the chart. As suggested by Lewis and Benedict, when the percentage of sugar in the blood is high, the final volume should be made 25 c.c. or 30 c.c., instead of 10 c.c., and the results, as given by the graph, multiplied by 2.5 or 5.0.

If it is desired to make duplicate determinations, approximately twice as much blood should be taken, 15 c.c. of picric acid added, and the volume made up to 25 c.c. Using 8 c.c. aliquots, as above, the results are readily obtained from the graph, if the weight of the sample is first divided by two. Similarly the graph is available for use with the original method in which 2 c.c. of blood are used, the value for the weight of the sample then always being 1 gram.

A METHOD FOR OBTAINING BLOOD FROM RABBITS.

The description of a method for collecting blood from rabbits, which has been in use successfully for several years in this laboratory may be a help to workers in other places. None of the devices employed present any features of extreme novelty, but the mode of their application appears not to be widely known.

The animal is fastened gently on the familiar "boot-jack" holder, which is shown in Fig. 2. Fig. 3 shows the animal in position on the board. When it is desired to take blood from the ear, the board is hung as shown in Fig. 4. After the fur is clipped from a portion of the ear over the vein to be used, preferably the marginal one, a small cut across the vein is made with a very sharp razor, and the blood is allowed to drop into a container. By this method varying amounts of blood may be obtained easily and quickly, and samples may be taken many times from the same animal. Recently we have taken samples from rabbits twice daily for six weeks.

The ear must be kept dry to prevent the blood from spreading on the surface, any clotting blood being removed with absorbent cotton. A good blood-flow may best be promoted by rubbing the ear vigorously before the animal is put on the board. The circulation may be further improved by bringing a lighted electric bulb near the ear. Toluene and xylene, widely recommended for use in producing local hyperemia, are too irritating for frequent application. Usually the bleeding may be stopped by pressing a small piece of cotton against the cut and holding it there a few seconds to allow the blood to clot; or an artery clamp may be used, though the clamp is avoided wherever possible, as

its use increases the difficulty of obtaining samples from the same place subsequently. Alcohol is used to disinfect the cut after the sample has been taken.

Fig. 4.—Rabbit in position for taking blood from ear.

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THE ROLE OF CHOLESTEROL IN PATHOLOGY*

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CHOLESTEROL occurs universally distributed in the plant and animal tissues, occurring in the former in an isometric form called phytocholesterol. Chemically it is classed in the sterol group of lipins. It was first isolated from gall stones in 1785 but its true, nonsaponifiable, fat-like nature was not discovered until 1814. When pure it crystallizes in white, plate-like rhombic crystals which are soluble in soap solutions; readily soluble in bile salts; and freely soluble in hot alcohol, hot or cold ether, carbon disulphide, chloroform, benzene and acetone. Its exact molecular structure is not known.[1] The following groups have been identified: a secondary alcohol standing between two methylene groups, a terminal vinyl group, and probably four saturated hexacarbon rings. There is one, or perhaps two, double bonds. It belongs to the terpenes and is probably closely related to the bile acids. Its empirical formula is $C_{27}H_{45}OH$ or $C_{27}H_{43}OH$. In general its chemistry is that of secondary alcohols. It readily forms crystalline esters many of which on cooling show a beautiful play of colors.

Quantitatively, it may be determined gravimetrically, or colorimetrically by recently perfected methods.[2] The gravimetric method is based on the reaction between cholesterol and digitonin which results in the formation of a precipitate sufficiently stable to be dried and weighed. The colorimetric methods are based essentially on the high color property of various cholesterol esters, the color values developed in unknown solutions being read by means of a standard colorimeter against that developed in a known solution. Colorimetric methods are admirably adapted to a study of the fluids of the body, but at best they are less accurate, giving slightly lower results than the digitonin method.

The physiological significance of cholesterol has been the subject of much controversy. Even now our knowledge of this subject lags sadly behind the knowledge of its pathological significance. The work on which the present views of the metabolism of cholesterol are based has practically all been done by one group of English investigators.

Of historical interest is Flint's[3] idea suggested in 1862 that cholesterol is an important factor in intoxications, especially in icterus. He claimed that the liver excreted cholesterol from the blood and that any disturbance of this function, resulting in bile retention, caused an accumulation of cholesterol in the blood with subsequent symptoms of toxemia.

Following him, Pages[4] in 1896 and Müller[5] in 1873, injected cholesterol into the blood stream with results which led them to confirm its toxic properties. Felz and Ritter,[6] in 1876, and Rywasch,[7] in 1888, repeated these experiments with opposite results establishing the view which has since been repeatedly confirmed, that cholesterol is non-toxic. In 1902 Kaiserling and Orgler[8] described under the name of "myelin" certain intracellular droplets in the normal adrenal cortex which differed from fat in being doubly refractive and in staining but slightly

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gray with osmic acid. In 1907 Panzer[9] and also Aschoff[10] showed these to be droplets of cholesterol esters. About the same time Lorrain Smith[11] and Dietrich[12] separately devised methods for the differential staining of these droplets and a little later Aschoff[13] and Kawamura[14] presented the first detailed morphological studies of them.

With the perfection of these methods for the morphological and the chemical study of cholesterol the work received a great impetus. As previously mentioned, one group of investigators addressed themselves to the physiological side of the problem. This work appears under the authorship of Dorie, Ellis, Gardner and co-workers[15] in a series of articles from 1908-1912 entitled "The Origin and Destiny of Cholesterol in the Animal Organism." Their earlier work suggested the following hypotheses:

1. Cholesterol is present in all cells, and it is not excreted as a waste product when the cell breaks down, but is resorbed and utilized in building new cells.
2. A function of the liver is to break down red blood cells and eliminate their cholesterol into the bile.
3. Cholesterol in the form of its esters is resorbed along with bile from the intestine.

Their later work supports these hypotheses and seems to warrant the following conclusions:

1. An increase in the cholesterol and cholesterol esters of the blood and liver directly proportional to an increased intake of cholesterol in the diet occurs in animal feeding experiments, but the kidney content is not affected.
2. Where food is withheld (inanition) there occurs an increase in the cholesterol content of the blood, liver and kidneys as well. In the latter the increase is due for the most part to cholesterol esters.
3. In the human a cholesterol balance can be established, i.e., the ingested cholesterol can all be accounted for in the feces, but the incidence of an acute infection with loss of weight disturbs this, yielding a positive balance in the feces.

Within the last year, Lander[16] reports results which prompt him to emphasize the ability of the organism to pick out and conserve cholesterol from a diet of an exceedingly low cholesterol content. The normal content of the various tissues and fluids will be considered at another place insofar as it bears on the pathological conditions to be studied.

Qualitative considerations with reference to the cholesterol content of the blood and bile are of interest in view of the light which they shed upon a function of the liver. The cholesterol content of normal blood is fairly constant (1.5 to 1.8 gms. p.m.) and is so distributed that the cellular elements contain largely free cholesterol while the blood serum contains both free cholesterol and cholesterol esters. The corpuscle content is affected but little by enriching the medium in which they float. It has been pointed out that while the blood on the whole is richest in cholesterol esters, the bile is richest in free cholesterol and that an increased content of the former induces a corresponding increase in the latter.[17] This would seem to suggest a specific enzyme for splitting cholesterol esters. Such a cholesterase has been described.[18] The recognition of the occurrence of cholesterol in pathological conditions dates back to the time of its preparation

from gall stones. From the standpoint of the morphologist a vast number of conditions have been known. A few of the more important ones only can be considered.

1. The so-called "pure" cholesteral gall stones range from 90 to 98 per cent of pure cholesterol. Their formation has variously been attributed to decreased alkalinity, decreased amount of bile salts, inflammation, colloid deflocculation and to an increased secretion of cholesterol with resorption of its solvents from stagnating bile.[19] The frequency of gall stones in women as shown by clinical and post mortem records[20] and the high cholesterol content of the bile during pregnancy are significant facts in view of the latter theory.

2. Cholesterol crystals occur in the tissues in a great variety of conditions, such as the atheromatous patches in blood vessels, encapsulated caseous areas, old infarcts and hematomas, dermoid cysts and, in especially large amounts, in the cholesteatomatous tumors of the ear and cranial cavity. LeCount,[21] in 1902, was the first to describe accurately the cholesterol giant cells so frequently seen in these conditions. Crystals also occur in old pleural effusions, hydrocele fluids, and in the cerebrospinal fluids, especially in tertiary syphilis.

Kaiserling and Orgler[8] on recognizing the anisotropic droplets in the normal adrenal cortex extended their studies to pathological conditions and identified them in amyloid kidneys, pneumonia exudates, retrogressive tumor and thymus changes, corpus luteum, etc. This condition has been called cholesterol ester fatty metamorphosis[22] and is especially prone to occur in the parenchymal cells derived from the urogenital anlage. Most conspicuous deposits occur in the epithelium of the "large white kidney," which are identical to the "protagone" bodies of the earlier histologists. This has also been spoken of as the setting free of the invisible cell lipin by autolytic processes akin to those giving rise to the initial cloudy swelling of true fatty degeneration.[23] There is some evidence to show that in the case of some organs, at least, an additional factor this latter is of exogenous or of endogenous origin, i.e., from the autolysis of plays a part.[24] This is a hypertension of cholesterol in the blood stream whether other organs.

Broadly speaking, then, deposits of cholesterol esters occur pathologically at the sites of low grade chronic inflammation, while deposits of cholesterol crystals occur under conditions of slow cell destruction in areas where absorption is poor.

The quantitative changes in the lipin content of the blood serum under pathological conditions have been the subject of much study[2, 25] from the standpoint of cholesterol and its esters.

Quantitative changes: (a) An increase of total cholesterol is found in arterial sclerosis, chronic Bright's disease, jaundice, diabetes mellitus, xanthoma, eclampsia, pregnancy, and obesity. (b) A decreased amount is found in pernicious anemia, all febrile diseases (except enteric fever), in tuberculosis, and in old age. The normal amount in the blood is placed at from 1.5 to 1.8 gms. p.m.

In arterial sclerosis associated with[26] chronic Bright's disease, there occurs from two to four grams p.m. while the content of the aortic wall is also greatly increased.[24]

In diabetes mellitus the increase appears to vary directly with the degree of acidosis and may be placed at from 3 to 4 grams p.m.[27] In hepatic disease with jaundice there is an increase varying with the degree of the jaundice but on the whole of less marked proportions than in the above conditions.

The blood analysis in xanthoma not associated with the above conditions, gives high values.[28] The fact that 70 per cent of such cases occur in women and particularly at the menopause vaguely connects the etiology with ovarian disturbances.

The increased cholesterol content of the blood during pregnancy is constant.[29] It is most marked during the last month. Values ranging from 2.5 to 4.25 gms. p.m. are given. An increased elimination occurs during the puerperium, chiefly through the milk, the hypercholesterolemia passing off the more rapidly in the nursing mothers. The blood of the newborn child is low in cholesterol content (1.19 gms. p.m.). The bile and adrenal content appears to parallel the blood content in these conditions of hypertension.

No explanations are offered to account for the hypocholesterolemia occurring in the above mentioned conditions. The suggestion that the cholesterol is used up in combating acute infections in an immunity reaction seems unwarranted. The positive balance of cholesterol[15] in the feces during acute infections and its increased storage in the reserve fat of old persons[30] deserve passing mention in this connection.

EXPERIMENTAL WORK WITH CHOLESTEROL.

Since the work of Ignatowski, in 1908, the question of the artificial production of artery lesions by excess feeding of certain foods has received attention.[31] Cholesterol has been identified as a food constituent producing this change. In addition to atheroma, cirrhosis and possibly kidney lesions are accredited to increased cholesterol ingestion.[8] So far such lesions have been producted only in rabbits, all experiments on carnivora being uniformly non-productive. It must also be added that atheroma and sclerosis are relatively frequent lesions in rabbits.

The relation of cholesterol to the growth of neoplasms has been studied in the case of experimental carcinomas in rats,[32] with results which seem to indicate that it accelerates the rate of growth and that it may be a factor in the incidence of cancer. This fact and the observation of an increased cholesterol content in the reserve fat of old people[30] may furnish a basis for the explanation of the increased incidence of cancer past middle life. With the cholesterol content of the tissues directly influenced by the diet,[15] in view of the preceding observations the question arises—can the incidence of cancer be decreased by decreasing the ingested cholesterol? Experiments have as yet failed to show this. In this connection, it is well to recall that the organism tends to accumulate and conserve cholesterol so that it is more difficult to decrease than it is to increase the cholesterol store of the tissues.[16] Examinations of the cholesterol[33] content of carcinomas in rats show that it is not affected by cholesterol injections; that it increases with the age of the tumor; and that the outside parts of the tumor mass contain less than does the central portion.

The part which cholesterol plays in phagocytosis and in immunity reactions

is very interesting and at the same time but little understood. It is well known that alcohol, chloroform and ether are detrimental to the natural defences against disease. They suspend phagocytosis both without and within the organism. This action has been attributed to their solvent power for the cell lipins in view of the fact that the administration of lipins tends to offset this effect within the organism. The experimental work[34] undertaken to show the relation of lipins to phagocytosis has been productive of results varying from inhibition on the one hand to increased phagocytosis on the other hand. This variety of results undoubtedly has arisen because *lipin mixtures* were used, i.e., the ether soluble tissue components, containing both lecithin and cholesterol which in this connection are antagonistic. Cholesterol even in small quantities vigorously inhibits phagocytosis but this inhibition can be completely overcome by lecithin.[36]

Under the heading of hemolysis, we must consider, separately, the actions of cholesterol and cholesterol esters. Cholesterol counteracts saponin hemolysis by chemically combining with the saponin, thus precipitating an inert cholesterol-saponin compound. In hemolysis by cobra venoms, cholesterol plays a somewhat different part. The hemolytic principle of cobra venom resembles an amboceptor in that it is complemented with lecithin[35] while the hemolytic action of the substance thus produced is very great, it is very markedly inhibited by cholesterol. Two explanations[41] are suggested. The action may be physical or chemical. The first assumes the formation of a definite compound, a "lecithid" which hydrolizes and yields lecithin. This lecithin is absorbed and carried down by the cholesterol as the unstable colloidal solution of the latter deflocculates. The chemical explanation assumes the formation of a definite chemical compound between the lecithin hemolysin combination and the cholesterol. Whether or not cholesterol plays a similar role in counteracting hemolytic bacterial toxins within the organism is not known. The question is of vital importance in all conditions associated with hemolysis. The administration of cholesterol is accredited with improving some cases of pernicious anemia.

Cholesterol esters do not inhibit cobra venom hemolysis.[41] There is evidence to show that cholesterol esters of unsaturated fatty acids may be a factor in furnishing complement for the cobra venom hemolysis.[35] These esters themselves may be directly or indirectly hemolytic. Bothriocephalus latus anemia is attributed to the cholesterol ester of oleic acid.[37] Various investigators have shown the hemolytic properties of unsaturated fatty acids.[38] In phosphorus and also toluylendiamin poisoning fatty acids have been demonstrated in salt extracts of the liver.[39] There is some dispute as to the efficiency of fatty acids as hemolytic agents. McPhedron[38] claims that the hemolytic activity is not determined by the degree of saturation. Lomar[38] believes differently and King[38] supports him.

In a series of clinical conditions associated with hemolysis, King studied the unsaturated fatty acid and the total cholesterol of the blood. He found an increase in the former and a decrease in the cholesterol. He connects this condition with hyperfunction of the spleen. He believes that this organ regulates the unsaturated fatty acid content of the blood. Its removal lowers the fatty acid concentration and raises the antihemolytic substances, i.e., total fat and cholesterol. On this basis then is explained the difficult hemolysis following

splenectomy as well as the reputed improvement in cases of pernicious anemia thus treated.[47] The striking resemblance in the clinical picture of severe bothriocephalus anemia and pernicious anemia should be recalled. On the other hand in simple anemia no such variations in cholesterol and fatty acid content occur. There seems to be a growing tendency to associate hemolytic lipins with the etiology of pernicious anemia.[48]

A striking example of endogenous hemolytic lipins is found in the "red degeneration" of uterine fibroids.[44] The amount of lipin in the ether extract of such fibroids varies directly with the degree of degeneration. From these tumors an active hemolytic agent has been isolated the action of which seems to be antagonized by normal serum. An excess of this hemolytic lipin acting on corpuscles in vitro produces first hemolysis without hemoglobin color changes, but later a brown precipitate settles, leaving a perfectly clean supernatant liquid. These results recall the physical explanation of the action of cholesterol in cobra venom hemolysis and suggest the spontaneous development of an endogenous lysin in retrograd focal tissue changes which not only affects the red cell stroma but also the cell proteins as is indicated by the color change produced in the hemoglobin.

The striking analogy between the inhibiting influence of cholesterol on the lecithin complemented cobra venom hemolysin, and the action of nonspecific antigen in complement fixation is apparent. Especially significant in this connection, are the recent observations which rank cholesterin enriched alcoholic tissue extracts as the most efficient antigen in the Wassermann reaction. Noguchi and Bronfenbrenner[49] contend that the antigenic value of the acetone insoluble fraction of the tissue extracts varies almost directly with its power to combine with iodine, i.e., with the amount of unsaturated fatty acids present.

Browning et al[41] show that cholesterol is more efficient than any of its compounds in enriching the alcoholic extracts for use as antigen. Walker and Swift[42] go still further in concluding that the cholesterin enriched alcoholic extract of heart muscle fulfills the requirements of a standard antigen in the syphilis reaction. With regard to the substances in the syphilitic sera which participate in the Wassermann reaction, it may be stated that while there appears to be a lipin increase in syphilitic sera,[45] no relation can be established between the lipin content and the antibody titer.[46] On the whole, the heterogeneous nature of the lipins taking part in this reaction prevents any conclusions as to the exact action of cholesterol.

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A REVIEW OF THE COMPLEMENT FIXATION TEST IN TUBERCULOSIS*

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FOR a great many years laboratory workers interested in tuberculosis have attempted to perfect methods of complement fixation for the diagnosis of this disease. The earlier work upon this subject was not a little discouraging because the results obtained were not uniform and were often out of harmony with the clinical analysis of the cases. Fixation reactions, therefore, had much laboratory interest but were of little value to the physician.

More recent investigations have been more encouraging and methods have been reported from a number of laboratories by which results sufficient to demonstrate the formation of circulating antibodies and often of great diagnostic value, have been obtained.

For the detection merely of the existence of tuberculosis in a patient, the physician has had at his disposal not only accurate clinical methods, but tuberculin tests of great delicacy. By complement fixation reactions it was hoped not only to establish additional diagnostic data, but by measuring the fluctuations of antibodies in the circulating blood, to throw light upon the activity of the existing disease and obtain prognostic information.

This review attempts to show how successfully this aim has been accomplished and along which lines of attack complement fixation in this disease has been planned and carried out.

From a study of the literature on the subject, it is clear that the principal difficulty consisted in procuring proper antigens. In the main, the general methods employed varied but little from the technic of the Wassermann reaction. The antigens however differed greatly and were as numerous as the different workers who devised and employed them. Almost all the tried antigens fall into four general groups.

Group I. Antigens in which the whole tubercle bacillus was used. This group includes antigens prepared from all the tubercle bacillus strains as well as from the allied acid-fast bacilli, i.e., smegma, butter bacillus (Rabinowitsch), bacillus lepræ, Grassberger's milk bacillus, and the Timothy hay bacillus. The bacteria were used in suspensions, living, and, after treatment, heat, cold, etc.

Group II. These antigens consisted of tuberculins. Practically all tuberculins have been made use of as antigens. Some have yielded good results, and in the hands of a limited number of writers the reports have appeared to be of great value.

Group III. This group comprises a large series of antigens which may be termed "tubercle bacillus derivatives or extracts." They represent many preparations obtained by subjecting the bacilli to various processes of extraction and digestion. The "partial antigens" of Much and Von Deycke are of this kind.

Group IV includes the antigens produced from normal and from tuberculous tissues, i.e., lung, kidney, brain, lymph-nodes, spleen.

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The following group tables indicate, though not completely, the number of antigens that have been employed:

GROUP I.—BACILLARY SUSPENSIONS.

NAME.	ANTIGEN.	RESULTS.
Caulfield and Beatty: Jour. Med. Research, 1911, xxiv, 122.	Bacillary emulsion.	Stage I.—33% positive. Stage II.—70% positive. Stage III.—62% positive. No fixation in normal cases.
Laird: Jour. Med. Research, 1912, xxvii, 163.	Watery emulsion.	4 positive out of 34 tuberculous patients.
Calmette and Massol: Compt. rend. Soc. de biol., 1912, lxxiii, 122.	Killed and concentrated bovine emulsions.	Results uncertain.
Fraser: Ztschr. f. Immunit., 1914, xx, 291.	Bacillary emulsion.	Positive: Tuberculous sera 1; Nontuberculous sera 4 Negative: Tuberculous sera 12; Nontuberculous sera 16 Luetic sera may give positive reaction.
Schultz: Ztschr. f. Immunit., 1910, vi, 709.	Bacillary emulsion.	Fixation in 5 out of 34 tuberculous cases.
Bang and Anderson: Centralbl. f. Bakteriol., orig., 1913, lxix, 517.	Bacillary emulsion, killed.	Reaction strong in positive cases; varies with severity of the infection. Tested cattle only.
Zweig: Berl. klin. Wchnschr., 1912, xlix, 1845.	Bacillary emulsion.	Fixation proportional to amount of infection. (Used active sera.)
McIntosh and Fildes: Lancet, London, 1914, ii, No. 8, 485.	Living fresh emulsions.	Pulmonary tuberculosis 76.7% positive. Surgical tuberculosis 80.7% positive. Syphilitic sera not fixed.
Radcliffe: Ibid., 488; Jour. Hyg., July, 1915, 36.	Bacillary emulsion living and freshly prepared.	Sputa positive pulmonary cases: Stage I 88.6%. Stage II 89.6%. Stage III 79.0%. Controls negative.
Loeffler: Deutsch. med. Wchnschr., May, 1913, 1025.	Bacillary emulsion, heated, dry.	Uncertain.
Stimson: Bull. Hyg. Lab. U. S. P. H. and M.-H. S., No 101, Aug., 1915.	Killed and living bacillary emulsions.	Maximum fixation appears in later stages. Too few cases tested.
Harris and Lanford: Jour. Infect. Dis., 1913, xiii, 301.	Suspensions of various acid-fast bacilli.	Demonstrated group fixing bodies, not specific for strains used.
Miller and Zinsser: Am. Jour. Med. Sc. (in press), Proc. Soc. Exper. Biol. and Med., 1916, xxiii, 134.	Killed or living, salt triturated.	

GROUP II.—TUBERCULINS OR FILTRATES.

NAME.	ANTIGEN.	RESULTS.
Ruppel and Rickman: Ztschr. f. Immunit., 1910, vi, 344.	Old tuberculin.	Fixation only in tubercular animals.
Armand, Delille, Rist and Vaucher: Compt. rend. Soc. de biol., 1913, lxxiv, 791.	Crude tuberculin.	100 cases tested. 4% positive fixation. 30% partial fixation.
Davidovics: Deutsch. med. Wchnschr., 1914, xl, 1, 21.	Old tuberculin.	Results uncertain; used active sera.
Besredka: Ztschr. f. Immunit., 1914.	Besredka tuberculin.	Stage I. Reaction always positive. Stage II. Reaction nearly always positive. Stage III. Reaction partial or negative. Luetic sera not infrequently fixed. Normal sera occasionally fixed.
Debains and Jupille: Compt. rend. Soc. de biol., 1914, lxxvi, 199. Ann. de l'Inst. Past., Apr., 1915.	"	Stage I and II (fair condition) 90.3% positive. Stage II and III (bad condition) 81.3% positive. 24% positive Wassermann sera gave positive tuberculosis fixation. Nontuberculous sera 17.3% positive. Normal sera 3.2% positive.
Kuss, Leredde and Rubinstein: Compt. rend. Soc. de biol., 1914, lxxvi, 244.	"	Positive in 89% of developed pulmonary tuberculosis. Not always negative with luetic sera.
Inman: Compt. rend. Soc. de biol., 1914, lxxvi, 251.	"	100 tuberculous sera tested; 95 gave positive reactions.
Bronfenbrenner: Arch. Int. Med., 1914, No. 6, 786. Ztschr. f. Immunit., 1914, xxiii, 2, 21.	"	Positive cases gave positive fixation 93.8%. Luetic sera gave positive 43%. Nontubercular sera gave positive in 18 out of 220 cases.
Renaux: Compt. rend. Soc. de biol., 1914, 864.	Besredka tuberculin, extracted with ether.	Avoids nonspecific fixation of luetic sera.
Petroff: (Not yet published.)	Petroff's tuberculin, potato broth.	High number of fixation in positive cases. Not infrequent fixation with luetic or inactive sera.
Craig: Am. Jour. Med. Sc., Dec., 1915, 781.	Besredka tuberculin, modified, alcoholic extraction.	Active tuberculosis fixation in 96.2%. Inactive tuberculosis fixation in 66.1%. Not positive in nontuberculous and luetic sera.

GROUP III.—BACILLARY EXTRACTS.

NAME.	ANTIGEN.	RESULTS.
Borissjak, Sieber and Metalinkov: Ztschr. f. Immunit., 1910, xii, 65.	Products of tubercle bacilli.	Antibodies are not specific for the antigens administered.
Dielman: Ztschr. f. Immunit., 1911, x, 421.	"Partial antigens."	Fixation in clinically nontuberculous cases.
Calmette and Massol: Compt. rend. Soc. de biol., 1912, lxxiii, 120.	A. Exobacillary dialysed products. B. Endobacillary products. 1. Water soluble. 2. Peptone water soluble.	Greatest number fixation with B-2.
Much: München. med. Wchnschr., 1912, lix, 685.	"Partial antigens."	Fixation in 77% of healthy people.
Kurt Meyer: Ztschr. f. Immunit., 1912, xlv, 359.	Deprived products of tubercle bacilli.	Fixation by the phosphatid derivatives.
Rothe, Bierbaum: Deutsch. med. Wchnschr., 1913, 644.	Watery extract of bacilli.	Strong fixation in a small percentage of tuberculous cattle.
Dudgeon, Meek and Weir: Jour. Hyg., 1914, No. 1, 72.	Alcoholic extract.	Maximum fixation in moderately advanced cases.
Stimson: Bull. Hyg. Lab. U. S. P. H. and M.-H. S., No. 101, Aug., 1915.	Calmette's B-2.	In 234 positive pulmonary cases 89% positive. Negative reaction is of little value.

GROUP IV.—TISSUE ANTIGENS.

NAME.	ANTIGEN.	RESULTS.
Hammer: Deutsch. Tierazt. Wchnschr., 1912, xx, 593. Münch. med. Wchnschr., 1912, lix, 1750.	Old tuberculin with extracts of tuberculous nodules.	43 positive reactions out of 46 tuberculosis cases.
Bierbaum and Berdel: Ztschr. f. Immunit., 1914, 21, 1-5, 249.	Bovine old tuberculin and extracts of tuberculous tissue.	Autopsy findings and sera reaction agreed in 65% of cases.
Wolff-Eisner: Wien. klin. Wchnschr., xxxvii, 1908, 1296.	Ground-up tuberculous lung tisue.	Results uncertain.

Considering the data compiled above, we observe that, except for Hammer's work, there is little to commend in the antigens of Group IV, and it may be contended that the tuberculin and not the tissues employed may have been responsible for whatever apparent value may be attributed to Hammer's antigen.

The antigens in Group III at one time occupied the attention of many

workers. It was maintained that to yield available antigen, the tubercle bacilli must be subjected to processes of chemical extraction. Von Deycke and Much, and also Much alone, described various split products of the bacillus, obtained after prolonged extraction with fat solvents. They obtained specific antigens from the bacillus, and they held that these specific antigens were contained in both the fatty acids and protein of the organisms. Their researches with these specific "partial antigens" failed however to bring about any particular advance in the usefulness of the fixation reaction. Kurt Meyer's extensive studies in producing tubercle bacillus derivatives, the watery extracts of Dudgeon and others, the peptone extracts of Calmette and Massol, the fermentation and autolytic bacterial products—all these types of bacillary extracts were employed with varying degrees of success.

It was hoped that the antigenic properties of the tubercle bacillus were contained in a particular chemical fraction of the bacterial body and that it would be possible to obtain this separately. Their experiments were formulated in the endeavor to obtain such an extract and prove its antigenic properties by injecting it into animals and demonstrating specific antibody formation in the animal serum by fixation against the original extract. These expectations have not been fulfilled.

In Group II we are dealing with tuberculins. As early as 1903 Bordet and Gengou[1] demonstrated fixation of complement with Koch's O.T. Soon Wassermann and Bruck,[2] then Citron[3] amplified these results in a limited series of cases. The many therapeutic tuberculins were investigated as antigens, and in addition numerous new tuberculins were proposed and tested.

Of recent years much attention has been focused upon the tuberculin described by Besredka. This is, briefly, a filtrate of an egg- meat-broth medium upon which tubercle bacilli are allowed to grow for several weeks. The filtrate only is employed as antigen. Good results with this antigen have been published by Besredka himself. Inman, Debains and Jupille have confirmed his findings. Renaux, Bronfenbrenner, and recently Craig have modified this antigen by extracting its lipins, and in this way seeking to avoid non-specific cross fixation with syphilitic sera. At best, however, the Besredka antigen shows a considerable margin of error. Craig, who reported no cross fixation and no fixation of non-tuberculous sera, obtained positive fixation in two-thirds of his clinically inactive cases, while Bronfenbrenner,[4] in his last publication obtained complete binding with the Besredka antigen in 20 per cent of syphilitic sera, and in a number of patients who had no tuberculosis, the reaction was positive.

More recently still, Petroff at Saranac Lake obtained good results by using for his antigen a tuberculin made by growing tubercle bacilli upon a watery extract of potato to which glycerine had been added. Here, too, there was not infrequently non-specific fixation with syphilitic sera.

The antigens in Group III then, have been in general available for the fixation test, but the reaction still remained of limited help to the individual practitioner because of its errors in fixing, occasionally, the sera of normal, non-tuberculous and luetic patients.

For final consideration there remain the antigens in Group I. These consist of tubercle bacillus emulsions or suspensions. McIntosh and Fildes, Dudgeon,

Meek and Weir, and Radcliffe, used saline suspensions of living or killed bacilli. Radcliffe, particularly, published strikingly good results emphasizing the advisability of employing young and fresh cultures. Courmont and Arloing devised defatted emulsions. Harris and Lanford tried suspensions of a variety of acid-fast bacilli related to the tubercle bacillus. In spite of much that has been written about the anticomplementary effects of the bacillary emulsions used as antigens, an impressive number of laboratory workers has encountered little if any difficulty in this regard. The English workers especially have found these suspensions very satisfactory. They claim a high proportion of positive cases, but Radcliffe reports that 74 per cent of clinically arrested cases also gave binding.

Debré and Paraff[5] proposed using tubercular exudates and transudates as antigen, titrating their antigenic properties against known, pooled tuberculous sera.

Recently Miller and Zinsser have employed an antigen prepared by triturating living or dead bacilli with dry crystals of ordinary table salt, then adding distilled water up to isotonicity. With this antigen the results have been as follows:

Of 232 clinically active cases with positive sputa, all but three gave positive fixation. One had an advanced pulmonary lesion; the disease in the other two was moderately advanced. Of ninety inactive or healed cases, eighty-three were negative. The reaction was positive in seven patients; five in this series had tubercle bacilli in their sputum shortly before the test was performed.* One hundred and forty cases were tested where the diagnosis was doubtful. In thirty-two the reaction was positive, and in some of these thirty-two cases the diagnosis of tuberculosis was subsequently established. Forty-three positive Wassermann cases gave no fixation with the tubercle bacillus antigen. Two cases were fixed with both antigens. In one the diagnosis of lues and tuberculosis was proved, the other case at present cannot be reached for investigation. One hundred and three non-tubercular cases were all negative.

From this rapid survey it will be seen that the bacillary emulsions, the tuberculins, and the extracts of bacilli, have yielded available antigens.

The filtrates of Besredka and of Petroff and the bacillary emulsion of Radcliffe, McIntosh have thus far served as the most available antigens.

The important factor has been established that in the fixation test we possess a method which will indicate for us the activity of tuberculous disease. The cutaneous skin reactions denote only a hypersusceptibility to the tubercle bacillus, and this state may persist even when the lesion is quiescent or healed.

With our own antigen the complement fixation in tuberculosis shows a remarkable degree of uniformity, in that practically all positive cases give positive fixation, and inactive cases are as a rule negative. Normal sera are always negative, and cross fixation with luetic sera is eliminated. The complement fixation test with this antigen can therefore serve as a measure for the control of the diagnosis of tuberculosis.

It seems that fair results have been obtained by a variety of methods. The

*Since the last published report (Proc. Soc. Exper. Biol. and Med., 1916, xiii, 136) we have learned from subsequent data that some of the cases with positive sputum classed as inactive, belong to the active group.

writer believes from his own personal experience that the antigen of Miller and Zinsser is at present superior to the others in use because it has so far failed to give cross fixation with luetic sera, has usually been negative in arrested cases, and has been almost invariably positive with active cases. It seems also to be the one most easily prepared.

However this may be, and the eventual decision can come only with great multiplication of comparative data, it appears from the many records, both our own and those reported by many of the workers cited above that complement fixation in tuberculosis is a definitely valuable diagnostic method which will occupy an important place among the practical methods of diagnosis.

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THE QUANTITATIVE ESTIMATION OF PHENOL-TETRACHLOR-PHTHALEIN EXCRETED IN THE FRESH BILE IN DISEASE OF THE LIVER*

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It was first shown by Whipple and Rountree that phenol-tetrachlor-phthalein injected intravenously is not excreted in any appreciable amount by the kidneys, as is phenol-sulphon-phthalein, but is excreted largely by the liver.[1, 2, 3, 4] It was shown, moreover, by Whipple, in experiments on dogs that artificial injury to the liver parenchyma, by chloroform or phosphorus poisoning, for instance, is accompanied by a marked diminution in the phenol-tetrachlor-phthalein output through the liver. Using the above preliminary experiments then as a basis for their work, Rountree and others in the same clinic concluded that by collecting all stools passed for forty-eight hours after an intravenous injection of 0.4 gm. of the dye, a fairly constant level of excretion for normal individuals was obtained, and that, moreover, the output was decidedly diminished in certain individuals suffering from disease of the liver. They concluded, therefore, that the test was of some value in diagnosis of such conditions.[2, 5, 6, 7]

The conclusions of Rountree and his co-workers as to the value of their test in diagnosis have not, however, been substantiated by others. Sisson, Frazier and Kana all reported it as useless in diagnosis.[8, 9, 10] As a result, therefore, of these contradictory reports, as well as of the fact that the technic of their test is rather cumbersome and time consuming, the test of Rountree and Whipple has been practically discarded.

During the course of certain work in which we have been engaged, we have had occasion to study a fairly large number of cases of cirrhosis of the liver. It

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occurred to us during this work that if after the injection of the phenol-tetrachlor-phthalein intravenously, we inserted a duodenal tube through the pylorus and, collecting all of the duodenal contents for a period of time by gentle aspiration, tested quantitatively for the dye which had been excreted in the bile, we might possibly obtain some information as to the excretory ability of the liver. Accordingly, a series of normal cases were first studied in this manner, the 0.4 gm. of phenol-tetrachlor-phthalein being injected according to the method described by Rountree and the contents of the duodenum being obtained during the ensuing two hours by continuous drainage. We hope that in this way two facts regarding the excretion of phenolphthalein could be ascertained: First, the time required for the first appearance of the dye in the bile, and, second, the quantity excreted in the bile during the two hours following the injection.

As will be noted from the following tables, the time required for the first appearance of the dye, after the intravenous injection, in normal individuals, varies from twelve to twenty-one minutes, while the amount excreted during the two hours following the injection varies from a trace to ten per cent of the amount injected. Moreover, it was found that the greater part of the two hour excretion occurred during the first hour after the injection, in normal cases.

After ascertaining the above facts regarding our normal cases, we have tried out the test, using the same technic upon a series of patients suffering from disease of the liver. The results of these tests were, briefly, somewhat as follows: Of five cases of atrophic cirrhosis of the liver (Laennec's cirrhosis) in well advanced stages, which were studied, the time required for the first appearance of the dye in the bile was somewhat extended as compared with the normal cases, varying in each case from twenty-eight to forty-five minutes, as compared with the time of from twelve to twenty-one minutes for the normal cases. The total two hour amount of bile collected showed, however, only slightly different percentages from the normal cases, three cases showing a practically normal output and only two varying somewhat from the normal, in that practically no dye was excreted in the bile which was recovered within the two hours. It was noted though, in those cases in which appreciable amounts of the dye were excreted, that practically all of the excretion occurred during the second hour as distinguished from the six normal cases in which most of the excretion occurred during the first hour.

In addition to the six cases of true cirrhosis, two cases of chronic passive congestion of the liver were studied in this way. None of these varied appreciably from the normal cases studied, however.

One case of extensive carcinomatosis of the liver was studied. In this case the initial excretion was decidedly delayed and practically none of the dye was excreted during the two hour interval, only a trace being found during the second hour.

This modification of the test has the advantage over the previous tests described by the originators of the method, that the dye is very easily estimated quantitatively in the duodenal contents, if the estimation is done promptly at the end of the collecting periods, the estimation being carried out in much the same way as in the kidney function test. There are several difficulties, however, which are met with in carrying out this test. One of these is that since the ejection of bile into the duodenum is apparently dependent upon the contractions of

the gall bladder, in the case of a supposedly delayed excretion time one may wonder if this is not due, perhaps, to a failure of the gall bladder to contract rather than to a really delayed excretion by the liver. Fortunately, however, there are certain simple procedures by which the contractions of the gall bladder can be, in a measure, controlled. One of the simplest of these, which we have found efficacious, is a simple change in the patient's position; from the side to the back, for instance. Repeated deep respirations associated with massage of the abdomen just under the right costal margin; the application of cold to the abdomen under the right costal margin; the causing of the patient to sit for a minute or so and then lie down again, will all often start up a sluggish gall bladder. Finally, the injection directly into the duodenum of from thirty to fifty cubic centimeters of a 0.5 per cent solution of hydrochloric acid[11, 12] will occasionally start contractions of a sluggish gall bladder.

Still another difficulty met with in the attempt to obtain constant results in these cases, is the fact that fear, nervousness and hunger seem to have a more or less marked effect upon the secretion of bile. A perfectly normal case may, for instance, on the first application of this test, excrete only a trace of bile during the whole two hours. This is usually, apparently, brought about by fear, as is shown by the fact that the subsequent tests will show a normal excretion both of bile and of phenol-phthalein. Such a failure as this happened to us in two cases, in both, however, subsequent tests were quite normal. Recently, we have hit upon a method which counteracts in great measure this very serious difficulty. This is the allowing of a fairly liberal diet to the patient for a few days preceding the test and at the same time the administration of sodium bromide in fairly large doses, and finally and most important, the administration of sodium salicylate for its chologogic effect for several days preceding the test. Our usual dose of the salicylate is fifteen grains three times daily for three

	DISEASE.	AMOUNT INJECTED.	1ST HOUR.	2ND HOUR.	TOTAL.	TIME OF APPEARANCE.
1.	Normal	0.4 gm.	4%	6%	10%	20 minutes
2.	"	0.2 gm.	Trace	6%	6%	25 "
3.	"	0.5 gm.	4%	1%	5%	20 "
4.	"	0.4 gm.	5%	Trace	5%	15 "
5.	"	0.4 gm.	4%	"	4%	18 "
*6.	"	0.4 gm.	Trace	"	Trace	21 "
*7.	"	0.4 gm.	2%	4%	6%	12 "
8.	Cirrhosis of Liver	0.4 gm.	Trace	3%	3%	30 "
9.	"	0.4 gm.	"	5%	5%	28 "
10.	"	0.4 gm.	"	9%	9%	45 "
11.	"	0.4 gm.	"	8%	8%	35 "
12.	"	0.4 gm.	"	4%	4%	32 "
13.	Chronic Pas. Cong.	0.4 gm.	2%	2%	4%	20 "
14.	"	0.4 gm.	4%	3%	7%	15 "
15.	Carcinoma of Liver	0.4 gm.	Trace	Trace	Trace	35 "

*Numbers 6 and 7 represent tests done upon the same individuals, No. 7 being the second attempt. The discrepancy in the results is probably due to the fact that the patient was considerably frightened on the first attempt, by the manipulations incident to the test.

days before the test is carried out. The difference in the amount of bile excreted by those patients who have had a preliminary dose of salicylate and those who have not is often quite striking.

Finally, as a result of this limited number of tests, we believe that the quantitative estimation of phenolphthalein in the duodenal contents is of little value from the point of view of the information derived, but that the excretion time of phenolphthalein after its intravenous injection is rather decidedly delayed in certain pathologic conditions of the liver.

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FOCAL INFECTION*

By John W. Shuman, M.D., Sioux City, Ia.

THE subject is one with which the general practitioner is constantly confronted; it is a subject which has gained considerable prestige among medical men during the past decade. Many of you have witnessed the quick subsidence of clinical symptoms, the speedy and sure cure of disease, in a case of so-called "rheumatism" following the removal of the cause—the focus of infection.

Although focal infection is not a new discovery, it is often newly discovered in a patient who has suffered an ailment for weeks, months or even years due to the lack of early suspicioning and recognition of the focus of infection by the physician. For many years John Ridlon advocated that many cases of chronic joint disease were not tuberculous joints. He states (personal communication) that "The first case I recall in this connection was a knee joint inflammation associated with and apparently dependent upon an attack of dysentery, in September, 1882. I immobilized the joint and cured the dysentery and the case rapidly recovered." At that time little was known of gonorrheal joint disease in its acute and chronic forms, the same was true of syphilitic joint disease.

We must examine the patient minutely from "hair to toe nails," overlooking nothing which will be of aid in diagnosing the etiological factor. Focal infection gives rise to many symptoms. Its main feature is pain of nerve, muscle, or joint; one, two or all three. This generally brings the patient to the doctor for relief. If the disease is acute a part or all of the cardinal signs and

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symptoms of inflammation about one or more joints may be present (polyarthritis). An increase of temperature, pulse and white blood cells may be noted. Deep abscesses of the teeth are a common cause and often are overlooked for the simple reason that they are not suspected. I do not wish to tire you with case reports, but here is a brief report which speaks for itself.

C. J., Dentist. Examined Jan. 3, 1916. Complained of morning frontal headaches. Lost 16 lbs. in four weeks. Acute and painful swelling of the right heel and stiffening of the left knee. T. 99, 2. P. 97. W. B. C. 8,000. B. P. (s) 180, (d) 115-20, mm. Hg. Left heart border well outside the M. C. L. A systolic murmer transmitted to the axilla was present. Urine; alkaline, 1010, albumin a trace and two granular casts present. The second upper bicuspid root was abscessed as also was the second upper bicuspid (right). They were extracted and two weeks later he had no headaches. B. P. (s) 148 (d) 100 and no systolic murmer. Urine acid, 1022, no albumin or casts. A month later B. P. (s) 145 (d) 95. I called him in yesterday and confirmed the cardionephritic condition. This case is but one of many which I have had the privilege to observe in which the kidneys were protesting in such a manner that a man would have to be very careless in order to overlook.

The tonsil as a disease focus is well understood. Too well perhaps, for the first thing a patient with a pain in a muscle, nerve or joint, is advised, even by his friends, is to have the tonsils removed. Therefore many an unoffending tonsil is wantonly sacrificed.

One point in this connection I wish you to consider is the advisability of removing the acutely inflamed tonsil, the focus of infection. If this is done in the instance necessitating such a procedure, prolonged treatment, chronicity of disease and complications will be avoided. The abdominal surgeon once advised "to wait" concerning acute appendicitis, if pus was a factor, until the pus had walled off or the attack was over before operating. Now all agree that the sooner and the more thorough the appendectomy the better it is for all parties concerned. I believed this to be the rational treatment of focal infection elsewhere situated.

Thorough chest and abdominal examination is demanded in every instance. In order that this may be done properly the patient should be stripped, and not examined through the clothing. It is difficult enough to ascertain correct findings through the skin, muscles and bone. Chronic pulmonary disease gives rise to neuritis, muscular pains and arthritis, but generally in a subacute manner. Fig. 1 is a röntgengram of a man who complained of severe pain and discomfort in the ankles, knees, elbows, and wrists. Compared with Fig. 2, a normal chest, it is not a difficult thing to note the wide variance. Fig. 2 shows the typical "moth-eaten" and mottled appearance of tuberculous species: Tubercle bacilli were found in the sputum. He had been sent to me for "Acute-rheumatic-baths," and not for a diagnosis which was too late,—he died.

The gastrointestinal tract affords several places in which pathogenic bacteria can culture and emit their toxins. The gall-bladder, appendix and colon are the principle ones. The diagnosis of infection, acute or chronic of any one of the three is a volume in itself and can only be mentioned. Here intestinal

stasis plays an important role. Abscesses of the fallopian tubes, in and about the rectum and kidneys are frequently foci of infection. Inflammation of the sciatic nerve, so-called "sciatic rheumatism" is not sometimes but always, secondary to a focus of infection, near or remotely situated. If this focus is not found there are the two old faithful alibis, trauma or the weather, for the lazy doctor to blame it upon. These excuses seem to never wear out. Let us dis-

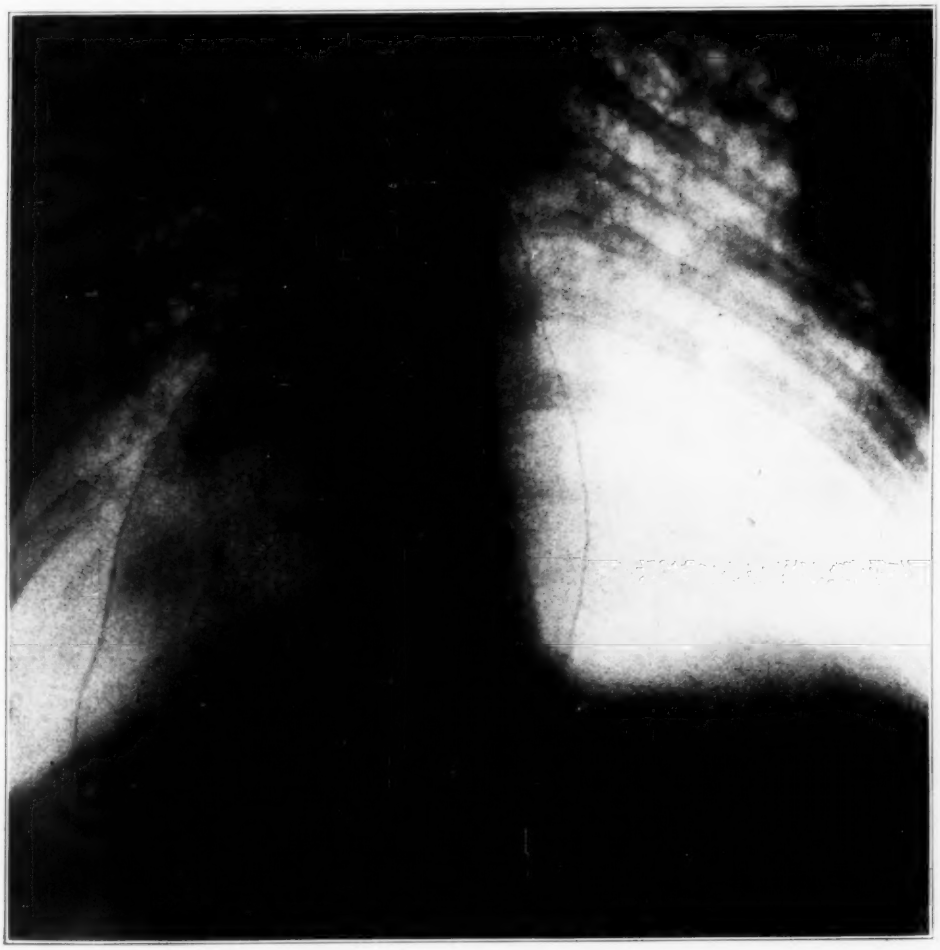

Fig. 1.—Mr. P. V. Heart normal. Lungs, bilateral tuberculosis. Note that both upper lobes are "moth eaten." This condition is also called "mottling." This case died two weeks later. Autopsy findings confirmed clinical findings and diagnosis.

card them. Let us begin our reasoning with a premise like this; there is no such a disease as rheumatism and strike the term from medical nomenclature. If we do so our conclusions will not be far wrong. If one careful search from scalp to toes does not reveal the trouble let us search again and again. If we cannot recognize it let us be fair and say we do not know.

Routine and methodical examinations are all very good but we must keep

our eyes open. We must not permit ourselves to be misguided as I was in the following case:

E. A., salesman, aged 26, complained June 20, 1915, of "articular rheumatism." January 5, of the same year, he had experienced a severe attack of polyarthritis and spent six weeks in bed. He stated that "tonsillitis always preceded the pain in the shoulder, knees and feet." Physical examination revealed submerged and diseased tonsils, temperature 99, pulse 110; urine—a trace of

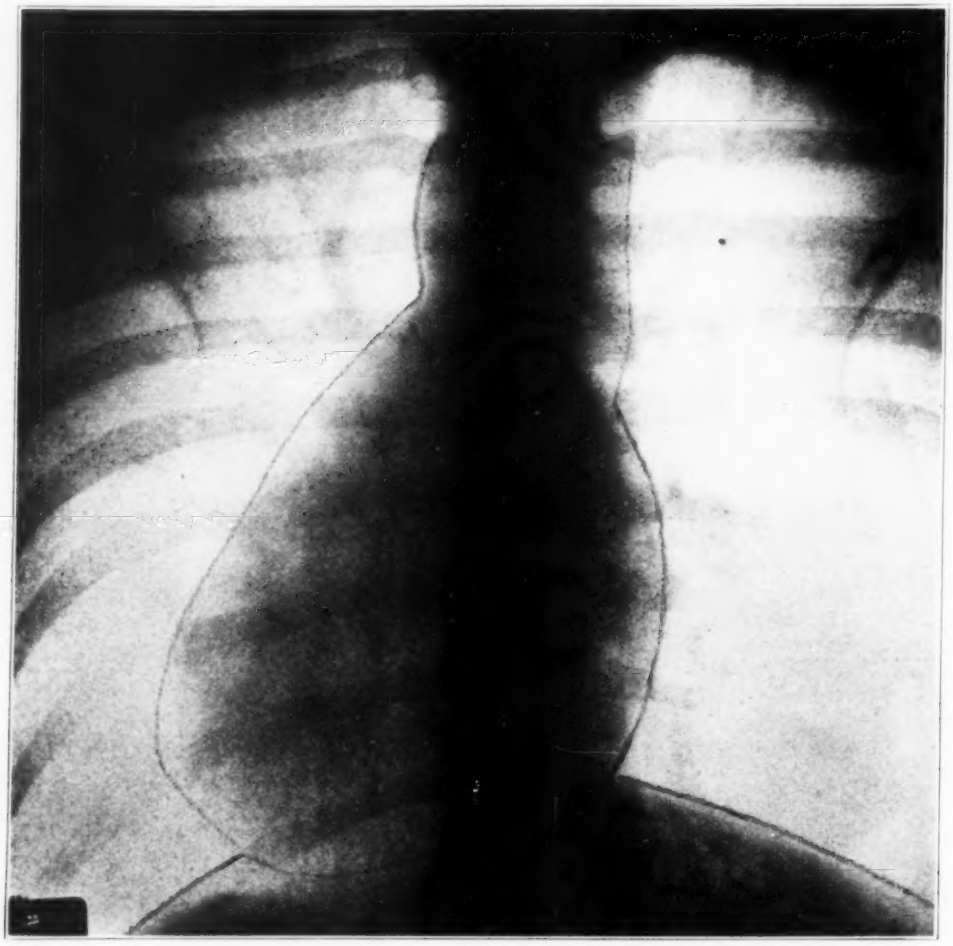

Fig. 2.—Mrs. S. Lungs and heart normal.

albumin and one or two granular casts. I could not make up my mind that the tonsils were the only cause of the trouble, so I went over him three times from "hair to toe nails" before I discovered an abscessed root of the left upper incisor, which he promptly stated "was one of long standing." Streptococci were found in the exudate. His dentist would not extract the tooth, but said he could save the root by treating it. This pleased the patient greatly. His tonsils were removed. Two months later an acute attack of arthritis occurred in his right knee; the "tooth" was still discharging. I took him to another dentist

and had that tooth removed; two days later the knee was well and has remained so ever since (April 26, 1916).

Focal infection in itself may be but a minor thing, but the complications are severe, oftentimes resulting in permanent injury and even in death. Hence the "rheumatic," syphilitic, and nephritic heart; the nephritis, cholecysititis, etc., etc. It is not reasonable that they were primarily affected. We now believe gastric ulcer to be infective in origin and that the avenue of infection is the blood stream; where did it come from, tonsil, tooth, lung or prostate? It is for the physician to find out.

A chronic running ear is but a little thing—perhaps you have seen them of long standing and the patient seemingly suffered little or no ill effect. I saw one once in a woman of 62 years. She stated "my ear has been discharging off and on for nigh onto thirty-five years," and she seemed to have no ill effects from it. Last January I visited a patient, who complained of her "spine hurting." Her people could not get the idea out of their heads that a fall in December was the cause of the complaint. She had been acutely ill but two days. Her physician thought a consultation unnecessary. Physical examination revealed marked tenderness over the right mastoid. T. 100. P. 90. Patient desirous of being undisturbed. She stated her "right ear had been discharging for sir years but had stopped running three days ago and had been paining some since." Spinal puncture and analysis of the fluid showed clearly that the patient was suffering from suppurative meningitis. The meningitis was undoubtedly secondary to suppurative mastoiditis which was secondary to an acute exacerbation of a chronic suppurative otitis media, the focus of infection, streptococci in origin; three days later she died.

There is a focus of infection I have not mentioned—abscesses of the skin and its integument. I have had the good fortune to diagnose and see operated eight perinephritic abscesses. In six of the cases a subacute or nearly healed furuncle or furuncles were found. In all six staphlococci were found in the pus from around the kidney. In one case there was a deep seated ulcer (traumatic) of the right thumb which was "scabbed-over;" staphlococci were found under the scab and in the perinephritic pus. The eighth case was secondary to a stitch abscess complicating an inguinal herniotomy. Colon bacilli were found in the pus of the stitch abscess and in the perinephritic abscess.

In conclusion; then let us make a thorough examination of our patients. Let us at the first opportunity afforded us suspect a focus of infection. If we can, let us find and eliminate the infection. If we cannot find it, let us be honest and say so.

A CASE OF PROGRESSIVE LENTICULAR DEGENERATION WITH AUTOPSY*

By Chas. Hunter, M.D., M.R.C.P., and S. J. Peirce, B.A., M.D.,
Winnipeg, Canada.

L. B., a girl of 18, sent by Dr. Swallow, of Russell, Manitoba, admitted to Winnipeg General Hospital on December 22, 1914, and died January 14, 1915, of erysipelas.

She was the third of a family of ten: four sisters alive and well, two dead, one at five and the other when two days old; one brother died at one year, one is healthy and the other, a man of twenty-four, began to shake and walk stiffly in the last six months. (This is confirmed by Dr. Swallow.) Patient said herself that "her brother was going the same way."

Father died of an accident at fifty-one; mother at thirty-eight, after a few days' illness; mother had always been "rather weak." Apart from the brother mentioned, no history of nerve trouble in the family.

Patient was always strong and healthy, and attended school until fifteen years of age when she left in the usual grade and began to help at home, both in the house and at outside farm work.

She menstruated only once, and then three years ago for one day only. Two years ago she noticed that she could not thread a needle or peel potatoes, owing to shaking of the fingers and hands; this shaking was present mainly when she tried to do such fine work and did not trouble her so much when she was busy at outdoor farm work. Over a year ago, she began to get stiff—noticed she had difficulty in getting into bed and in turning in bed, owing to the stiffness. She remarked, too, that her mouth began to keep open in spite of herself and that at times the saliva would dribble, while her friends noted that she smiled a great deal and that for over a year, her speech was indistinct—they often told her to speak clearly. For some months she had needed help in dressing and undressing; the stiffness was worse in the morning; she could walk around with ease, but owing to the shaking which had increased and the stiffness, she could do very little work in the house—only sweeping the floor or scrubbing, and that with increasing difficulty. Recently, in stepping backward, she found she had difficulty in checking the backward movement and sometimes continued to move backwards till held up by the wall. A year ago, she had an attack of measles, and was in bed then for a month, owing to "weakness." The shaking was less on getting up. No dyspeptic symptoms at any time and never any difficulty in swallowing.

CONDITION ON ADMISSION.

She was a well built and very well nourished young woman, with full bust development and pubic and axillary hairs. Lungs normal. Heart normal but rather rapid (80-92); no anemia; leucocyte count 9,600 (polymorphonuclears 68%, lymphocytes 26%, large mononuclears 6%); urine normal; gastrointestinal track negative; no evidence of cirrhosis of the liver; test breakfast gave free

*From the Winnipeg General Hospital.

Fig. 1.

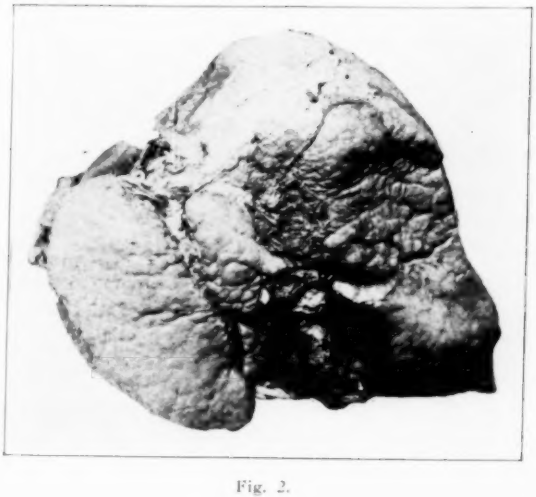

Fig. 2.

hydrochloric acid 45 and total acidity of 70; Wassermann on blood doubtful (2.0.0.0.0.) Temperature on three occasions before erysipelas developed, reached a little over 99 degrees.

Her facial expression was striking and is well shown in repose in the accompanying photograph (Fig. 1). The mouth was constantly open, upper incisors and canines visible with a little of the gum, upper third of front lower teeth also visible with the large tongue very slightly protruding and occasionally slight dribbling. She smiled very frequently and often without any cause; then the mouth opened a little wider, the upper teeth and gums much more exposed and lower teeth less so. The smile lasted long. Apart, however, from this change around the mouth, the facial expression was absolutely fixed and reminded one constantly of the expression in paralysis agitans. The upper eyelids drooped as shown; there was, however, no increase of the drooping as the day went on, as in myasthenia gravis.

CRANIAL NERVES.

Vision and optic discs normal. Pupils equal, medium, reacting to light and accommodation. Ocular movements normal. No nystagmus in any direction.

Corneal reflex brisk on both sides; no sensory impairment over the face. The patient could close the mouth, but did so slowly and relaxed rapidly. She could not whistle.

Hearing was normal. Palate moved well and symmetrically; palate reflex present. Swallowing unimpaired and no difficulty at any time admitted.

The sternomastoids were always hard and did not relax even when she was lying still; she could move the head only slowly from side to side, often with tremor of the head and arms—there was marked stiffness of the muscles of the back of the neck.

The large tongue was protruded slowly but only a little way and the movement was accompanied by slight tremor of the tongue and lips.

Speech was indistinct and monotonous; some days it was moderately distinct, but on other days one had frequently to ask her to repeat what she said.

MOTOR SYSTEM.

The muscles were everywhere well developed and possibly a little firmer than usual to palpation. She walked with ease and with only a trace of rigidity in her gait; no ataxia; Romberg negative. When standing still, however, and pushed gently backwards, she could not recover herself but would continue to move backwards, sometimes till held up by a pillar or wall some distance away. With practice in the hospital, this symptom improved considerably. In startling contrast to her freedom in walking, was her extraordinary helplessness in bed—she turned round with the greatest difficulty and often stopped when half-way from supine to prone position in a helpless, appealing way. She assisted herself in turning with her hands on the head of the bed. Similarly, when getting into bed and rising up from bed, her movements were slow, helpless and rigid in the extreme. The movements of the spine, when she was up, were free: she could bend forward and laterally with ease and this combination of freedom of movement when up, with apparent rigidity on turning in bed forcibly struck one, suggesting hysteria.

When exhibiting the patient at the Hospital Clinical Society as a case of progressive lenticular degeneration, I was disconcerted, on asking her to lie down on the floor and to get up, to find she could do so with comparative ease, rising, however, from the hard floor like a pseudohypertrophic patient. I found later she was aware that she had little difficulty in turning on, or getting up from, a hard floor as opposed to a soft bed.

She needed to be helped to dress and undress while in the hospital; she could put on and take off her clothes, but only extremely slowly, with marked rigidity and some tremor.

A certain amount of rigidity seemed everywhere noticeable in the muscles of the extremities—e.g., in the lower limbs, in flexing and extending or rotating the thigh and in the upper extremities, in flexion and extension of the elbow and in pronation and supination. All movements could be actively and passively carried out—there was no contracture, though she tended to carry the hands slightly flexed at the wrist-joints and extension of the hands at the wrist-joint was definitely weak. The rigidity was slightly more marked in the left upper and lower extremities than in the right.

The muscles of the abdominal wall were quite relaxed, when patient was lying in bed and the abdomen could be palpated with comparative ease.

Generally, no tremor was noticeable; on excitement or on muscular effort, a marked rhythmical tremor developed, mainly in the left upper extremity, though when marked, involving the head in flexion and extension, the left ankle and toes in flexion and extension, and slightly the right upper and lower extremities in a similar fashion to the left. The tremor particularly developed when she tried to put on or to take off her stockings and was well marked in the finger-nose test. Protrusion of the tongue developed a slight tremor of the tongue and lips. In the left upper extremity, where the tremor was most marked, it took the form of alternating flexion and extension at the elbow, flexion and extension at the wrist and alternating pronation and supination to a slight degree; its range varied with the muscular effort and excitement and sometimes reached *2″—3″*. Patient could control the tremor sometimes when ordered to do so; at other times, the command aggravated the condition.

SENSORY SYSTEM.

No alteration in any form detected and no subjective sensation.

REFLEXES.

The abdominal reflexes on the left side were never elicited, though frequently examined; on the right side, the upper abdominal reflex was elicited repeatedly but never the lower. Knee-jerks and achilles-jerks brisk and equal; no ankle-clonus, though a pseudoclonus was repeatedly obtained. A definite extensor response was elicited repeatedly from the sole, particularly on the left side, but more frequently the response was of the usual flexor type. Sphincters normal.

During her three weeks' stay in the hospital, patient answered questions intelligently and took quite an interest in the other patients, who remarked on her frequent smile.

She was quite cheerful but remarked repeatedly that the doctors had not

helped her so far. The nurses thought she was rather simple and childlike.

On January 11 she developed facial erysipelas, which spread rapidly over the head, her temperature rose rapidly to 105 degrees and before death to over 107 degrees; sensorium clear but progressive cardiac weakness—death on January 14, 1915.

Autopsy was performed January 15, 1915, by Dr. Peirce.

SYNOPSIS OF POST MORTEM FINDINGS.

External Examination.—Body of young adult female, well developed and well nourished. Upper portion of face edematous. Bleb-formation on lower portion of forehead, left ala of nose and lobe of right ear.

Internal Examination.—The only organs that show changes other than are incidental to septicemia are the liver, spleen, and brain.

Liver.—Markedly cirrhotic. This is best seen on the lower surface which

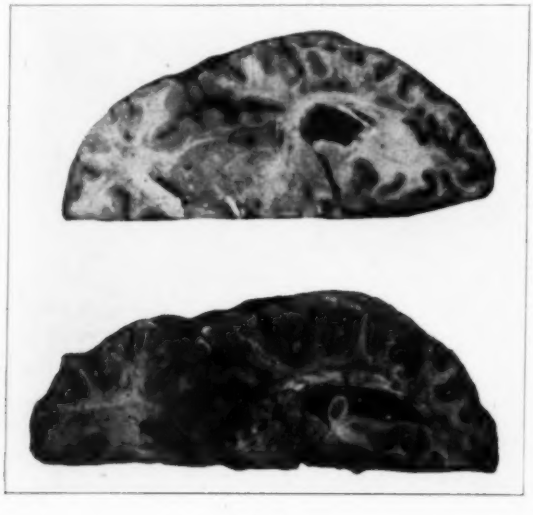

Fig. 3.

presents a multitude of small lobules 2-10 mm. in diameter, separated from one another by bands of connective tissue. Organ is somewhat smaller than normal and of firm consistence. Weight—1,402 grams. (Fig. 2.)

Spleen.—Uniformly enlarged, soft, but pulp is not defluent. Weight 355 grams.

Brain.—Cerebrospinal fluid somewhat excessive in amount. Vertex of right hemisphere shows a thickening of the arachnoid, which appears as a milky area about one inch in diameter. Extreme anterior poles of both hemispheres show single, symmetrical, cord-like adhesions of arachnoid to dura.

Weight after hardening in ten per cent formalin:

Left hemisphere	639	grams.
Right hemisphere	650	"
Cerebellum and pons	167	"
Total	1,456	"

Horizontal section along a plane immediately below the extremities of the corpus callosum (Pierre Marie's coupe d'élection) gives the appearance shown in Fig. 3. The figure below is that of the left hemisphere of the brain. The figure above is for comparison, and is that of the left hemisphere of the brain of a boy who had died of tuberculous meningitis. The only noteworthy feature of the section is a uniform diminution in size of the lenticular nucleus. The reduction in size seems to be confined largely to the putamen. The normal pattern of the cut surface here is blurred by a brownish foggy appearance which extends outwards into, and to some extent obliterates, the external capsule. The nucleus presents no cavitation nor does it appear softened. The cortex appears everywhere of normal thickness. Gross serial sections reveal no other abnormality.

Spinal Cord.—Beyond old adhesions of the meninges no gross change can be made out.

Microscopic Examination.—Section of the lenticular nucleus (Van Gieson and Ehrlich's acid hematoxylin) show a dearth of nerve cells, and an increase in glial elements. The nerve cells that persist show mostly an eccentric nucleus and brown granules in the cytoplasm. The vessels show no perivascular infiltration and no endarteritis. Small mononuclear cells containing brown granules are fairly numerous.

Section of Cord.—(Osmic acid). Show no demonstrable areas of degeneration. Cells of the anterior horns appear normal. Meninges show no infiltration.

Sections of the liver confirm the gross appearance. There is great increase in connective tissue elements. In many places the liver substance is broken up into isolated islands of liver cells by bands of connective tissue. Many liver cells show fatty change.

Remark.—No change was found microscopically in the internal capsule or in the pyramidal track in the cord to account for the occasional extensor response and the constant absence of the abdominal reflexes on the left side.

These physical signs presented a difficulty in regarding the case as one of "Progressive Lenticular Degeneration," as described by Kinnier Wilson in "Brain" in 1912.

LABORATORY METHODS

RADIOGRAPHIC STUDIES OF CEREBRAL VASCULAR LESIONS. A PRELIMINARY NOTE*

By Charles O. Dhonau, Cincinnati, Ohio.

MUCH has been written concerning the vascular lesions in the cerebral vessels and very many of the usual photographs have been used to illustrate cerebral lesions due to vascular disease, but so far as I have been able to discover no previous attempt has been made to study the vascular system of the brain as a whole by means of injection and x-ray photographs. This is perhaps more to be wondered at because in studies of the vascular system of the heart the method has been of considerable value, and when it is recalled that the brain suffers at least as frequently as the heart from diseases of its vessels it would seem that in this viscus the same methods would be at least of equal interest. It is, perhaps, the fact that the technical difficulties in injecting the brain after it has been removed from the skull are greater than in most other organs. There are so many collateral communications which must be broken in removing the organ, that the injecting fluid or mass leaks out in innumerable places. The waste is therefore great and in dealing with injection masses which are satisfactory for x-ray work artifacts are very easily produced and these are often misleading. Therefore, the method which was worked out by me involved leaving the brain *in situ*. By this method the brain could be thoroughly washed out, then injected readily, and finally removed with little danger of leakage provided a proper mass was used and other necessary precautions were observed.

The matter of the injection mass gave a certain amount of trouble. A successful mass is one which must form a fairly stable suspension and which will, after fixation of the brain, be impenetrable to the x-rays. Jamin and Merkel[1] in injecting the coronary arteries used red lead wax and turpentine as one mass, and red lead iodipin and wax or gelatine as another. A third mass was composed of a heavy proportion of red lead in a 10-15 per cent solution of gelatine. Experimentation with these masses showed in the case of the red lead, wax and turpentine, that constant agitation of the mass was necessary during the entire injection. The experiments with gelatine were as a whole unsatisfactory on account of the effects of temperature on the viscosity of the mass. While in the case of single removable organs these masses were utilized in a satisfactory manner, the injection of a circulation as extensive as that of the brain from a point quite distant, as in the case of my method, required an injection mass which was stable and which was not affected by variable temperatures.

Hauch,[2] injecting the renal arteries, used 500 grams of red lead, 500 grams of paraffine oil and 250 grams of turpentine oil. Experimentation with this mass showed that it had to be agitated during the injection to prevent an uneven con-

*From the Pathologic Institute of The Cincinnati General Hospital.

centration of the lead. The plates made from the organs in which this mass had been injected were fairly satisfactory. On account of the inherent weaknesses of both the Hauch and Jamin Merkel masses for injection, I experimented with the injection mass suggested to me by Dr. Herbert A. Brown, Curator of the Museum of the Pathologic Institute of the Cincinnati General Hospital. This was a suspension of litharge in glycerine. This proved to be a perfectly homogenous mass and in the proportion of 225 grams of litharge to 500 c.c. of glycerine penetrated into the principal medullary branches of the cerebrals. This mass threw an even shadow and the plates showed a clear delineation of the arteries from the Circle of Willis to the larger branches of the terminal cerebrals.

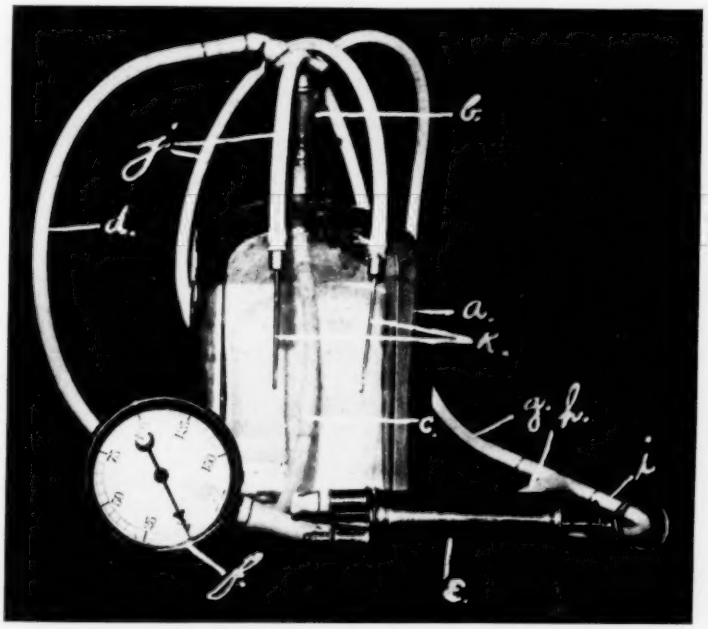

Fig. 1.—The injection apparatus. (a) a 64-ounce squat injecting bottle; (b) a rubber stopple into which was fitted a metal gooseneck; (c) a rubber tube extending from the gooseneck to the floor of the bottle; (d) a rubber tube extending from the gooseneck to a Y connection; (e) a hard rubber aspirating and injecting pump; (f) a pressure gauge; (g) a rubber tube from the gooseneck to a Y connection on the air side of the apparatus; (h) a rubber tube from the pressure gauge to the Y connection on the air side; (i) a rubber tube from the air pump to the Y connection on the air side; (j) 2 rubber tubes from the Y connection on the injection side to the canulæ; (k) 2 metal canulæ.

The technic of the injection was as follows: The right and left common carotid arteries and the right and left internal jugular veins were brought to the surface at the upper margin of the sternum. Canulæ were introduced into both carotids. Incisions were made into both internal jugulars for drainage. The external carotids were ligated. An injection of 2,000 c.c of one per cent solution of borax was made simultaneously into both carotids. The injection of the borax solution cleared the blood from the arteries and sinuses with the exception of such blood as was in the form of thrombi. The washing was followed by an injection of the litharge-glycerine mass. This last injection was started at low pressure and was continued until the maximum resistance was noted. Maximum resistance in this form of injection equaled a pressure of 18 pounds to the square inch. The amount of the mass injected before the point of maximum resistance was

reached averaged 350 c.c. After the injection was stopped, the canulæ were removed from the carotids and the arteries were ligated. The head was placed in a position of retraction during the entire injection. Immediate cranial evisceration was then done by the usual method practiced at postmortems. On incising the scalp the injection mass exuded from the arteries. On retracting the scalp the temporal arteries were found fully injected. On removing the calvarium (which was done without injury to the brain substance) the meningeal arteries were found fully injected. On removing the dura the terminal branches of the cerebrals were found fully injected. To prevent the escape of the injection mass the internal carotids and basilar artery were ligated before division. The viscus was then removed from the skull and was placed in a specimen jar containing a 10 per cent solution of formaldehyde and sufficient cotton to give

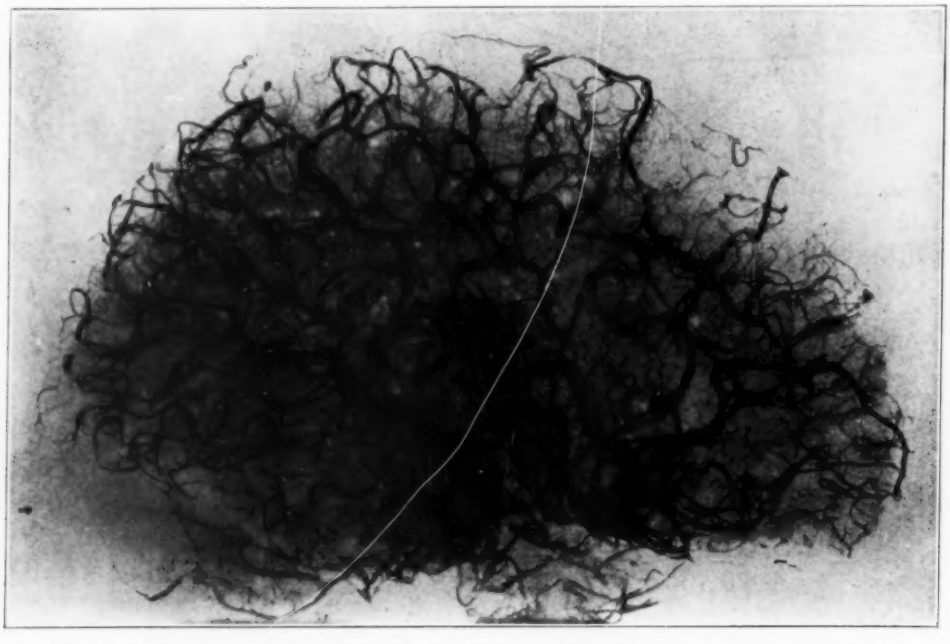

Fig. 2.—This is a normal specimen in which there is no vascular disease. Note the regularity of the arteries as compared to those in Fig. 3.

support to the brain. After an immersion of 48 hours it was removed from the specimen jar and the exposure to the x-rays was made. Two exposures were made. The first was of the entire brain with the base toward the plate. The second was of both hemispheres with the mesial surface of each toward the plate. With both hemispheres on the same plate the lateral view was found to be of much value on account of presenting both a normal and an abnormal specimen for differential study.

To illustrate the results of this method, photographs of two plates are presented (Figs 2 and 3).

There was a clinical diagnosis of cerebral hemorrhage. The autopsy diagnosis made without sectioning the brain was arteriosclerosis; diffuse subarachnoid hemorrhage from the right and left posterior cerebellar arteries; throm-

bosis of the right and left posterior cerebellar arteries. After the exposure to the x-ray the brain was sectioned, showing, a mass of clotted blood which entirely filled the left lateral ventricle; an area of white softening in the left hemisphere corresponding to that portion of the medulla which is supplied by terminal branches of the ascending frontal artery. There were no pathologic changes in the right cerebrum. The x-ray diagnosis was arteriosclerosis, the vessels showing marked tortuosity and irregularity; rupture of the left middle

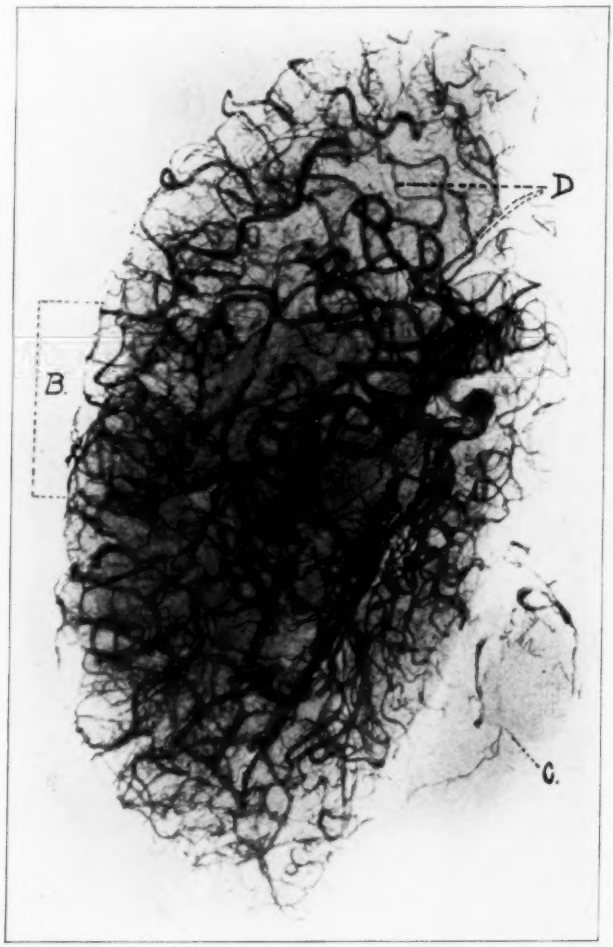

Fig. 3-A.—Case No. A-2997.

cerebral artery at a point in its main trunk, as shown by an apparent projection into the intima of this artery of a part of the clot, when seen stereoscopically; thrombosis of the posterior cerebellar arteries, as seen by the absence of the injection mass in these vessels; thrombosis of the terminal branches of the ascending frontal artery on the left side, as seen by the absence of the injection mass in these vessels; possible multiple miliary aneurismal formations in the right cerebrum. To demonstrate these findings, I have marked the print as follows:

(A) Area of thrombosis of the terminal branches of the ascending frontal branch of the left middle cerebral; (B) Corresponding normal area in the right side; (C) Thrombosis of the right and left posterior cerebellar arteries; (D) Possible miliary aneurismal formations.

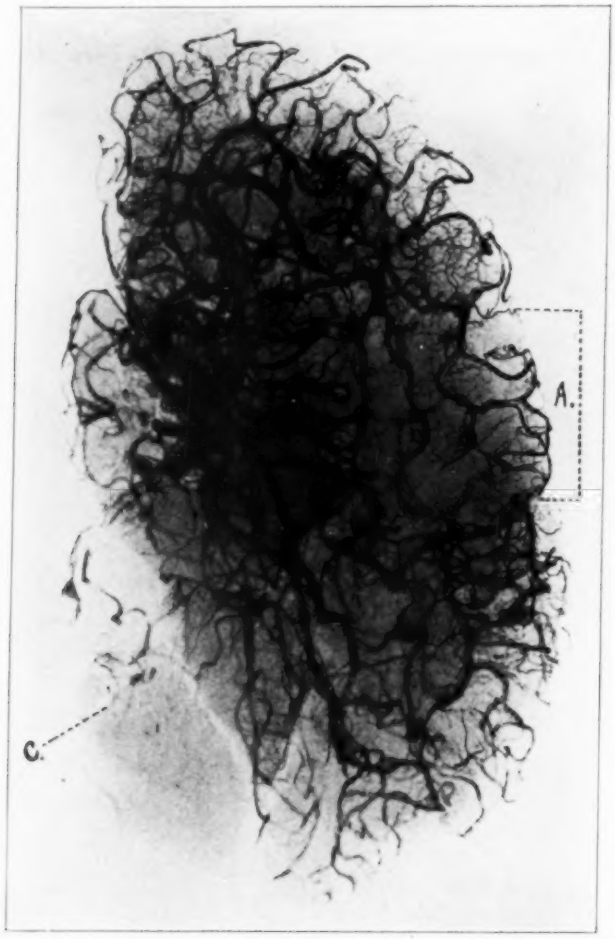

Fig. 3-B.

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[2]Hauch: Fortschritte der Rontgenstrahlen, Band XX, Heft 2, Hamburg, 1912.

ardel[3] reports a death from choking, in which attempt the skin showed no marks. There were extensive hemorrhages in the deeper tissues, and two similar cases have been observed by Messerer.[4]

The hemorrhages found on dissecting the throat of one murdered in this way are more extensive than those after either hanging or cord strangulation. They usually lie in and between the muscles, in the tissues of the thyroid and in some cases in the submaxillary salivary glands. In nineteen cases Reuter found hemorrhages, in eleven in the cervical muscles, and especially in the tissue of the thyroid, four times in the tonsils and pharynx, and seven times in the larynx. Laceration of the muscles has not been reported, but, as has been stated, fracture of the cartilages is frequent. The fractures, however, are more common in the aged, and in the newly born they may not, and often do not, exist. Fracture of the hyoid bone is rare and the trachea is not often injured. Patenko[5] has studied the mechanism of fractures of the larynx in choking and concludes that in some persons ossification of these cartilages begins as early as 35 years of age and may progress to such an extent that fracture is relatively easy. It must not be inferred that fracture of the larynx in the young may not be caused by choking and such a case has been reported by Stolfer[6] in a youth of 15.

With the exception of the unique case reported by Binner[7] there is no known instance of suicide by strangulation with the hands. When the larynx is compressed by one's own hand, the hand loses its grip before death is reached. The case reported by Binner is interesting but is doubtful. A woman who was known to have tried to choke herself was found dead in a squatting posture by the side of her bed. Her elbows rested on her knees and pressed against the side of the bed. Her hands formed a fork in which rested her throat while her head hung forward and downward.

This form of strangulation being always homicidal is often combined with other injuries to the body. The assailant knocks his victim down or pushes him over, then with his knee on the chest he proceeds to strangle him. The pressure on the chest may cause a bruise or an abrasion and not infrequently fracture of one or more ribs. Again, the assailant wounds his victim by a blow on the head or a stab in the chest and then strangles him while lying in a helpless state. In some instances strangulation with the hand is completed with a cord, or death having been caused by the former the murderer may fasten a cord about the neck of his victim to make it appear that he has suicided. The differential diagnosis between strangulation by the hand and by the cord is easy when the external marks are distinct and distinctive, otherwise quite difficult. Reuter is of the opinion that the more profuse extravasations of blood after death by the hand and their locations, especially in the tissue of the thyroid, the submaxillary glands and the tonsils, are sufficiently characteristic to justify a diagnosis. They certainly are if the external markings are confirmatory, otherwise they are not. There are several reported cases in which the murderer has suspended the corpse of his victim in order to awaken the suspicion of suicide, but fortunately the differential diagnosis between hanging and strangulation by the hand is generally quite easy and certain. In the first place a comparison of the external marks about the throat is usually sufficient, but these

failing, the differences found on dissection of the throat are likely to be satisfactory. Fracture of the annular cartilages and of the thyroid and large blood extravasations in the soft parts of the throat are very rare in death by hanging, and indeed, occur only with a long drop, while they are practically common and certainly are typical in strangulation by the hand. Strassmann reports a case of a woman found hanging in an open sling of soft cloth. The external throat marks were not distinctive, but dissection showed double fracture of the annular cartilages, fracture of the superior bone of the thyroid and four hemorrhages in the mucous membrane of the larynx. After these findings he did not hesitate to testify that death had been induced by strangulation with the hand and not by hanging. Wilhelmi[8] was able from the presence of fractures and their nature to show that a woman who had been found suspended had actually been strangled by hand, and in this instance the body was not examined until it had been in the grave for five and one-half weeks.

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—*V. C. V.*

The Nephroses

THE term nephrosis is an inclusive one. It includes all the diseases (except tumors) from which the kidneys suffer, from mere edema to the true inflammations, whether suppurative or nonsuppurative. It therefore includes what we speak of as nephritis, or Bright's disease.

The term nephritis includes two conceptions: one of them clinical, the other histological, or anatomic. Clinically it means the presence of certain qualitative or quantitative urinary variations, which are, generally speaking, increase or decrease of the total amount of urine and the presence of albumen and casts in the urine. In the anatomic sense it means this and something more. It means inflammation, which is to say the kidney is the seat of certain vascular and perivascular changes which we associate with a very distinct histologic picture, in which vascular dilatation, congestion, and exudation are in the foreground. Albumen and casts in the urine may be produced by any process which disturbs sufficiently the nutrition (or metabolism) of the renal cells. Inflammation is one such process. Mere local acidosis, which produces edema without the signs of inflammation is another.

There are three main types of renal lesions, the simple nephroses, including simple edema and simple necrosis; the inflammatory nephroses or nephritides; and the disuse nephroses, including the arteriosclerotic and renal atrophies.

THE SIMPLE NEPHROSES.

This group is one which is fundamentally the most important. In it are included all those types of renal disturbance which are the result of partial or

complete lack of oxygen, from the edema of the athlete, and that of the soldier on the march, both of which are benign and transient, to the sudden complete necrosis of infarction. In it belong the so-called "alterative nephritides" of certain texts, and the toxic necrotic conditions which are the result of the action of organic or inorganic poisons. In all these albumen and casts appear in the urine; in all the quantity of urine is diminished. The main difference that needs mention in discussing these simple nephroses, is that in the kidney of edema, the organ may return to a completely normal condition very rapidly; in the toxic degenerated and in the infarcted kidney the chances are that this does not occur, but that an increase of scar tissue is formed as a part of a secondary inflammatory process. In the case of infarcts the scarring is localized; in cases of certain chemical intoxications it is diffuse.

It may be interesting in connection with these simple nephroses to recall Osler's remark, that "every case in which albumen is present must not be called acute Bright's disease, not even if tube-casts be present. Thus the common febrile albumenuria, although it represents the first link in the chain of events leading to acute Bright's disease, should not be placed in the same category."

THE ATROPHIES.

Similar in some respects to the simple nephroses are the atrophies. These, like the former, depend for their urinary phenomena upon lack of oxygen, but the cause, in this instance, lies not in the composition of the blood, but in the condition of the blood vessels, which have been so changed by one or another factor, that the blood, qualitatively perfect though it be, does not reach the cells in sufficient amounts to keep them at a physiologically active level. What happens to the kidney in arteriosclerosis of a simple type, is exactly what would happen to the kidney of a moderate simple edema, provided the edema were chronic, which is to say, provided the circulatory conditions remained the same. In the renal edema of the sprinter the oxygen supply in the blood is not quite sufficient for the cells of the kidney. In the arteriosclerotic kidney, the oxygen in the blood is sufficient but it cannot reach the cells rapidly enough. The result of this decreased access of the cells to oxygen is that they undergo atrophy, become smaller, and some of them disappear, or, if the progress of the vascular disease is somewhat more rapid, they undergo degeneration and disappear, and in vanishing leave the stroma of the organ apparently more abundant, relatively increased, and this tends to produce the appearance of sclerosis or fibrosis in the organ. Actually such an atrophic organ contains no more fibrous tissue than a normal organ,—it merely possesses less parenchyma.

THE NEPHRITIDES.

This group of the nephroses is characterized, so far as the renal parenchyma is concerned, by all the anatomic changes which are present in the former groups, and by certain other features, which are clearly inflammatory. In all the types one phenomenon dominates the picture, and that is the one we term exudation which may or may not be associated with fibrosis. It is from the obviousness of the associated fibrosis that we are led to speak of an acute or

a chronic inflammation. Fibrosis in the absence of exudation does not spell inflammation though it may be a sequel of it. A sclerotic kidney is not necessarily one of interstitial nephritis.

It has been customary for many years, and will undoubtedly remain customary for a very long time, to speak of parenchymatous and interstitial nephritis, by which is meant types of inflammation which affect the parenchyma of the kidney, or the intestinal tissues as the case may be, predominantly. One goes further if he follows the texts and divides the nephritides into other types, such as simple, hemorrhagic, and suppurative; and as glomerular, tubal, and diffuse; and further, as acute and chronic. These terms are, however, merely helpful in characterizing nephritides from the standpoint of general extent. The process in all the cases is the same. It varies only in the extent to which it affects the kidney, in the type of exudation, and the rapidity of its development. Moreover the inflammatory process always starts in the neighborhood of the blood vessels, and therefore it happens that at but one place in the kidney can an inflammation be spoken of as being primarily parenchymatous,—that place is the glomerulus. All other forms of inflammation are essentially interstitial in type, and the peculiar and sometimes characteristic changes which one observes in the parenchyma are secondary ones due to disturbances of metabolism resulting from poisons which reach the cells from foci of inflammation, or from lack of oxygen which is the result of perivascular conditions. There are times when these secondary changes dominate the picture; when the parenchyma has undergone fatty or soapy degeneration, as, for example, in the "large white kidney." Under such circumstances we have been in the habit of speaking of a chronic parenchymatous nephritis, a designation which we may preserve if we wish, provided we realize that it is not that, but a type of interstitial nephritis with abundant secondary degeneration—a *diffuse nephritis.* This is very reasonable, because if we study the other classical type of kidney, the "small red" one we find similar changes, though not so evident. In this the focal inflammatory fibrosis dominates the picture, and obscures the parenchymatous changes. Perhaps it were best to say that in all nephritides parenchyma and interstitial tissues are both affected, but that in one the degenerative changes predominate; in the other the productive. Perhaps it is best to speak of *diffuse* and *focal nephroses.* All of these remarks apply equally to the suppurative and tuberculous kidneys. In these the only difference is that the lesions tend to be more obviously and completely focal than even in the interstitial form, and therefore they acquire specific characteristics.

This would all be very simple were the forms of nephroses simple, which, as a rule, they are not. As a rule they are combined, at least this is apt to be almost completely true except in cases in which death has been produced suddenly by trauma. That the conditions existing in the kidneys may undergo sudden changes is shown in many clinical cases. For instance, a person who has a well developed interstitial type of nephritis does very well for long periods of time. The vascular and perivascular changes have advanced to such a stage that the compensatory powers of the organ are quite limited. Much of the parenchyma has disappeared, but the portion which remains is physiologically active and produces a normal amount of fluid, with little or no albumin and

few or no casts. If at such a time he meets a sudden traumatic death, the kidney would appear as a "small red kidney," a primarily contracted kidney. But let him suffer an attack of enteritis; let him absorb a few more colon bacilli; a too large quantity of a poison. He dies in uremia (cerebral edema) and the kidney at postmortem is apt to be gray and then we are apt to say that it is a "secondarily contracted kidney," or small gray kidney. And it is quite probable that under such conditions unless he makes use of the clinical details the pathologist may insist that what he finds tells the whole story.

It would seem then that for the sake of simplicity the following classification would be serviceable:

A. The diffuse nephroses—(acute intoxication).
Simple edema, and necrosis.
Acute diffuse nephritis (acute Bright's).
Glomerular nephritis.
Hemorrhagic nephritis.
Chronic passive congestion.
Chronic diffuse nephritis (chronic parenchymatous nephritis).
Glomerular nephritis.

B. The focal nephroses—(vascular or infective).
Infarction.
Arteriosclerosis.
Acute interstitial (septic or simple) nephritis.
Chronic interstitial nephritis.

C. Combination of focal and diffuse forms.

Such a classification has one value if no other; namely, that it allows of a better understanding of the urinary findings,—that is to say, it permits the clinician and the pathologist to stand a little closer together.

URINARY CHANGES IN THE NEPHROSES.

I. Diffuse nephroses—(acute and chronic).
Decrease of urine.
Casts present.
Albumen in very noticeable amounts.

II. Focal nephroses—
a. Acute—
Urine may be decreased, depending upon the number and extent of the lesions.
Casts present.
Albumen in moderate amounts.
b. Chronic—
Little or no decrease of urine.
Few or no casts—these come from the diseased foci.
Little or no albumen.

III. Combination Forms—The urinary changes vary.

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—*P. G. W.*

Diabetic Therapy

IT has been a usual comment of those who have watched the development of scientific knowledge in this country, that it is particularly in the application by practical methods of principles discovered elsewhere that the American investigator excels. In certain of the medical sciences this comment is particularly applicable, for it has been by developing in a practical way the fundamental discoveries of European investigators that the American surgeon has acquired so world-wide a fame. The development of surgical technic has been going on for many years, but it is only recently that a similar advancement can readily be recognized to be under way in the domain of internal medicine, and in no branch of this is it more conspicuous than in the therapy of diabetes. It is now many years ago since Bouchardat, Naunyn and Cantani first suggested that severely diabetic patients should be starved until the urine became, if not entirely, yet very nearly sugar-free, but the suggestion was not generally adopted, because it had meanwhile come to be clearly recognized that the coma to which many such patients succumb is in some way related to the appearance in the urine of the so-called acetone, or acidosis, bodies, and that the amount of these often increases alarmingly before the starvation had got well under way. The starvation method of treatment had not been developed sufficiently, nor had its beneficial influence on the course and outcome of the disease been clearly enough demonstrated to make the majority of physicians feel justified in adopting it to any great extent. Acting partly on the suggestions offered by the experience of European clinicians, and partly because of results obtained by observing animals rendered more or less diabetic by partial removal of the pancreas, Allen undertook the thorough trial of starvation in specially selected cases of diabetes kept under constant observation in the wards of the Rockefeller Hospital. He showed among other things that in the great majority of even severe cases the acidosis which may develop during the first few days of starvation afterwards subsides, so that in a surprisingly short period of time the urine contains neither sugar nor more than small quantities of the acidosis (acetone) bodies. Sometimes, however, the treatment is not so successful. When the ability of the blood to combine, without disturbance of its reserve alkalinity, with all of the acids produced by the faulty

condition of metabolism—of which the appearance of acetone bodies in the urine is an indication—is studied in diabetic cases, a thoroughly reliable criterion is furnished of the effect of the treatment.

The technic of several of the laboratory methods employed in securing these data has already been described in this journal, and for the present we would direct attention to a recent communication by Woodyatt in which the general principles underlying the development of acidosis are admirably set forth, and an appreciation of which will enable us to explain why the starvation causes acidosis bodies to appear in some cases and not in others.

For the thorough combustion of fat in the animal body a certain amount of carbohydrate must be simultaneously burned. Fat evidently is a less readily oxidized foodstuff than sugar; it needs the fire of the burning sugar to consume it. If the carbohydrate fires do not burn briskly enough, the fat is incompletely consumed; it smokes, as it were, and the smoke is represented in metabolism by the acidosis bodies. Such a closing down of the carbohydrate furnaces may be brought about either by curtailment of the intake of carbohydrates, as in starvation, or by some fault in the mechanism of the furnace itself, as in diabetes.

Fundamentally, therefore, acidosis is due to the same cause in starvation and diabetes, namely, to an improper adjustment between the metabolism of fat and carbohydrate. Besides fat, portein may also contribute to the production of acidosis bodies when carbohydrate combustion is depressed. Bearing these principles in mind, it is easy to see how the intensity of acidosis which develops during starvation will depend upon the relative metabolism of carbohydrate on the one hand and of fat and protein on the other; it will therefore depend on the amounts of these foodstuffs which have been stored in the organism, and this again will depend on the nature of the diet previous to the starvation period. For the first few days following entire abstinence from food in a healthy, well-nourished individual, very little if any acidosis bodies will be excreted in the urine, because the carbohydrate stored in the body as glycogen has sufficed during this time to maintain the proper proportion between fat and carbohydrate. Afterwards, however, the appearance of acidosis bodies is to be expected, because the glycogen stores become exhausted long before those of fat. If starvation be still further prolonged, a stage will come when the fat, as well as the carbohydrate, is used up so that the organism has now to subsist on protein alone. When this stage arrives, the acidosis will diminish, for although from certain of the amino acids of which this is composed acidosis bodies might be derived, yet this does not actually occur, because a large part of the protein molecule (nearly half) also becomes changed into dextrose, which by burning, as above explained, prevents the formation of acidosis bodies from the other part of the molecule. For the same reasons, marked acidosis will not be expected to occur during any stage of starvation in lean persons, who from the start must utilize mainly their stored protein to supply the fuel upon which to live.

In diabetes exactly the same principles apply, but to an organism in which the ability to metabolize carbohydrate has been depressed, so that "the maximum rate at which dextrose can be oxidized is fixed at some level which is absolutely

lower than in health." Therefore, since a certain proportionality must exist between the rates of combustion of fat and carbohydrate, the diabetic can thoroughly oxidize less fat; in other words, an amount of fat which could readily be burned in a healthy body is improperly burned by the diabetic, and acidosis bodies accumulate.

"In order to check a diabetic acidosis, it is necessary to restore the proper ratio of fatty acid to dextrose oxidation," which can best be done by starvation, rest in bed and warmth. But this treatment may not at first suffice, because we have to deal not only with the acidosis bodies derived from fat but with those which can be derived from protein on account of the diabetic organism having lost the power even of burning the dextrose which is derived from this foodstuff. By persistence in the starvation, however, the ability of the organism to utilize carbohydrate usually becomes so far restored that enough burns to prevent acidosis. Every case of diabetes cannot therefore be expected to react in the same way to starvation, the determining condition being the relative quantities of glycogen and fat stored in the body at the outset of the fasting period, and this again on the nature of the previous diet.

To sum up, "fasting will lower acidosis either in health or in diabetes if it has the effect of stopping a one-sided metabolism and throwing the tissues on a more nearly balanced ration of fatty acids and glucose." A practical point may be noted here, namely, that there is likely to be more danger of serious acidosis developing during starvation in the case of fat than in lean diabetics.

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—*J. J. R. M.*

Chloroform Necrosis of the Liver

THE fact that chloroform may produce important changes in the organs of the body, particularly in the liver, is a significant one which is based upon considerable clinical and experimental observation. That ether and other narcotics are less apt to produce the same changes is also well known. The essential of chloroform poisoning is severe intoxication associated with anatomical changes which resemble those of acute yellow atrophy, phosphorous poisoning, eclampsia, certain septicemias, and acute syphilis.[1] These changes have been supposed to be the results of local defective oxidation in the affected organs. Wells believes that the chloroform damages the liver cells in such a manner that while their general activities are lost, the intracellular proteolytic enzymes are not interfered with and so produce autolyses. The intoxication results from incomplete hepatic function.

In connection with this matter of chloroform necrosis, Graham[2] brings apparently conclusive evidence that in chloroform poisoning "the liver necrosis

[1]For general account of these conditions see Wells' Chemical Pathology, 1914, p. 49 et seq.
[2]Graham: Jour. Exp. Med., 1915, xxii, p. 48.

is produced chiefly by the action of acid (largely probably by hydrochloric acid which is formed in the metabolic destruction of chloroform), and this ability to produce liver necrosis is a general property of the alkyl halides, all of which probably yield halogen acids in their breakdown in the body." Graham calls attention to the ease with which chloroform produces necrosis as compared with ether, and to the probability that that difference depends upon either different molecular effects or to different products which are formed in the metabolism of the two substances.

When chloroform is oxidized, the reaction results in the formation of phosgene and hydrochloric acid:

$$CHCl_3 + O \rightleftarrows COCl_2 + HCl$$

When phosgene in the presence of water reacts to form carbon dioxide and hydrochloric acid,

$$COCl_2 + HOH \rightleftarrows CO_2 + 2HCl$$

It is evident then that when chloroform is oxidized in the presence of water, 3 molecules of hydrochloric acid are produced, for each molecule of chloroform present.

With these facts as a starting point, Graham studied the tissue changes produced by hydrochloric acid, by chloroform and other substituted products of methane and by other alkyl halides. He has found that while certain narcotics produce these changes, others do not, and that all the substances, narcotic or otherwise, which do cause them are those which on splitting give rise to halogen acids. Certain substances such as chloral, which resemble in a general sort of way chloroform, produce no necrosis because they do not produce, on oxidation, the proper acids. Moreover, the activity of the substances in producing necrosis runs parallel with the amount of halogen acid produced during metabolism. For all of these statements there is abundant experimental support, even for the presence of acid in the areas of necrosis.

Graham wisely insists that the production of halogen acids is not the only factor in the production of necrosis, but that they are important ones. He calls attention to the fact that when disturbances of oxidation result, other acids (organic) are apt to accumulate—notably lactic acid, and this acid is able to produce profound tissue changes. It is possible that in phosphorous and arsenic poisoning, for instance, it is the organic acids, which accumulate because of decreased oxidation, that cause the anatomic changes. In chloroform poisoning, or that produced by the halogen narcotics, he seems to prove that the halogen acids are the essential factors.

Graham has not only made this contribution but has followed it up therapeutically and shows that the administration of alkalies is able to inhibit the production of the lesions. He used the alkali-salt solution of Fischer. He says in conclusion,—

1. After the administration of some of these drugs, there has been noted

an increase of neutral salts of the halogen acids in the urine, a fact which indicates that the corresponding halogen acids must have been formed somewhere in the body.

2. The necrosis-producing powers of dichlormethane, chloroform, and tetrachlormethane parallel the amounts of hydrochloric acid which these substances theoretically can yield in their breakdown outside the body. Likewise, the power to produce tissue changes exhibited by the ethyl compounds, varies directly with the ease with which they form their respective halogen acids *in vitro*.

3. Ether and chloral hydrate do not yield halogen acid in their breakdown in the body likewise do not produce necrosis. They induce only edema and fat infiltration to a less marked degree.

—*P. G. W.*

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STUPID WORDS AND STATEMENTS COMMONLY APPEARING UPON LABORATORY REPORTS

By B. G. R. Williams, M.D., and E. M. Williams, B.S., Paris, Ill.

A SLIP of the tongue is often overlooked, but a slip of the pen is regarded as inexcusable. A laboratory report, standing as it does as a permanent record of precise data and not to be altered save by amendment provided by re-examination, should be strictly accurate.

Yet to study or even glance many reports, the reader is often led to doubt if the examination was really worth while. The family physician desires a usable record of diagnostic worth. Hoping for this he has sacrificed time and patience in collecting and preparing the specimen. The sick man has provided the "good iron dollars." The laboratory man, intensely interested in the problem, has made the examination and has determined (to his satisfaction) what, if any, light it throws upon the diagnosis. Yet, the permanent record raises innumerable questions, and finally leads those who have not seen the work carried out, to doubt the value of the examination and to discredit laboratory work in general. No matter how skilled the examination, it frequently happens that the written report is valueless.

THE EQUIVOCAL "NEGATIVE" AND SOME OTHER WORDS.

A test may be positive or negative, but these terms, and especially the latter, are much misused. The odor of a urine is rarely negative, the conclusions on a sputum examination (embracing as it should many tests) should never be stated by a terse "negative" and a differential blood count is never negative. Negative is an adjective and implies the absence of certain qualities. It may be used in complement-fixation work, for example, to show that specific antibodies or complement-binding qualities are absent from the patient's serum. But it seems to us that its use to express the fact that a color is normal, that the worker is unable to find tubercle bacilli in a sputum (pus, blood and other important findings may be present) and its promiscuous use on clinical reports, is not justified.

The word "precipitate" (noun) is often used incorrectly by men who should set a standard for accurate, scientific expressions. Truly speaking, coagulated albumin is not a precipitate but is a coagulum, the solid or semisolid state of a sol. The term "precipitate" should be restricted to the solid state of a crystalloid as thrown from true solution.

It is our observation that "mucus" and "mucous" are quite generally confused or spelled incorrectly.

"Blood," "hemoglobin," "hematin" and "erythrocytes" should be more carefully differentiated in laboratory reports. This criticism is not unjust, for it may have some bearing upon the diagnostic use of the information.

The term "found" is not strictly incorrect, but in chemical work "detected" is perhaps preferable whereas in microscopy the term "identified" seems to us

to be more desirable. It is likewise merely a question of preference when we advise the use of the words "sensitive test" rather than "delicate test."

"Trace" is not definite enough, for what one worker may consider a trace, the next will report as absent. The latter may report as a trace that which the former would regard as a small amount. "Trace" should always be qualified as "questionable trace," "appreciable trace," "marked trace," and "more than a trace." This suggestion is volunteered because the average practitioner is inclined to disregard the word "trace" and dismiss it as significant of no clinical meaning.

"Present" may be permissible in some portions of the report but is not definite enough as a rule. It seems to us that it is not sufficient to report casts as "present," but these casts should be classified and an estimate of the number per field should be made. The reason is obvious: a few hyaline casts may have but little meaning, but where a dozen or more granular casts are present in every portion of the drop the case is different. Moreover, it may be possible in a series of examinations to approximate a prognosis. Elsewhere we have shown that by such series of examinations, vascular nephritis may be diagnosticated by the study of the urine alone.

REPORTS OF URINALYSIS.

Amount.—While every laboratory worker would prefer to know the actual amount of urine being passed each twenty-four hours, it is not always possible to secure these figures. The blank which provides for this, should not be filled in with the word "negative," but should be marked "Amount not stated." If it is probable that the amount is normal, but there is uncertainty because figures are not available, the sentence "Probably normal, according to patient's statement" or "Probably normal, as suggested by other properties" will leave no question in the mind of the reader.

Color, odor.—We doubt if the color of a urine should ever be stated as "normal," but concede at the same time that it is very difficult to find a satisfactory word to express the color. It has been very difficult for laboratory men to agree upon a chart or scheme of normal and pathological urinary tints. Moreover a color which might be normal for a twenty-four hour specimen of 48 fluid ounces, would by no means be normal for one of 10 fluid ounces or 100 fluid ounces. The color of the sample may be unimportant or absent but in either case the word "negative" should be avoided since it has become ambiguous through usage. The same principles apply to the designation of the odor of the urine. We fear that the man doing much urinary work is becoming a bit careless in the use of words which may mean much to him, but mean nothing to the family physician or scientific readers in general.

Solids.—Total solids should always be qualified by reporting as "per twenty-four hours" or "per liter."

Reaction.—It seems to us that where litmus is not used as the indicator, that the name of this indicator should be stated with the results.

Albumin.—The more progressive laboratories are now reporting albumin as serum albumin in order to discriminate between it and other albuminous bodies. We routinely report it in filtered sample, but further qualify if it seems

to be explained by pus, blood, and so on. We eliminate the question of bacteria by refusing to examine decomposed samples, by pointing out this source of error in the report or by shaking with calcined magnesia, filtering until clarified and controlling the tests carefully. When we fail to detect serum albumin in a sample of urine, we do not use the term "negative," but state that it is absent by such and such a test or by the routine clinical tests. It seems to us that the term "albumen" should be dropped.

Other Chemical Tests.—"None" or "O" is usually employed by us to show that Bence-Jones body, bilirubin, glucose and so on are absent.

Inasmuch as the purpose of the laboratory report blank is to serve as a reminder as well as a convenient means for classifying the findings, it seems that it is well to provide space for all tests of known value. In every examination, however, it is not necessary for the worker to set up diazo, pentose and urochromogen tests. In such case it is not proper to fill in these blanks with the word "negative" (and this is being done by some men), but it is much better to draw lines through these words, showing that the respective tests were not made. The laboratory man is not called upon to fill every blank in the report sheet, and is unfair to himself and his clientele if he attempts to do so.

Quantitative Work.—In the same connection comes the question of how much quantitative work the laboratory man carries out in the routine urinalysis. Most men estimate the solids, if provided with twenty-four hour figures, and some calculate the urea. Well and good, but a large number likewise fill out all the blanks in the quantitative section—not with figures, of course, but with the words "present" or "absent." What stuff is this? Many of our laboratory men (and we judge by their reports) find urates, chlorides, sulphates and phosphates in samples of urine submitted to them for diagnostic examination. Perhaps these same men ofttimes wonder why practitioners lay so little emphasis upon the value of their reports. We will not be surprised one of these days to find water reported as present in some urinary sample.

Microscopy.—The laboratory man is at his best in reporting microscopic findings. The practitioner has learned this, and often instructs him to make only the microscopic study.

However, there is one point which must be straightened out eventually and this perhaps will be realized only by a general agreement. This is in regard to the reporting of pus cells or the various types of white blood cells in the urinary sediment. Show us the report that states that pus cells were absent, and we can usually prove that the worker was either careless and not thorough in his examination or that he possessed intelligence enough to ignore the few leucocytes he saw that the family physician might not be confused thereby. Here are some rules in this connection which are not to be taken as final, but are designed to open up the question anent the reporting of pus cells:

1. The presence of many of these cells (at least two in every high power field, unless found by low or high power in clumps of five or more) should be reported not only as such, but the point should be emphasized that "this urine contains true pus—case of pyuria."

2. If possible, distinguish between small renal cells and the various types of white blood cells, and so state upon the report.

3. Attempt to determine the cause and origin of the pus not only for the diagnostic value of such a procedure, but to actually determine in a given urine where there are a few or more than a few white cells, that these are truly pathological and that pyuria should be reported.

4. Never report cancer cells in a urine sediment. Malignancy is determined, not by cell types but by cell relations. Cells desquamated from a benign papilloma may present a vegetative appearance but may not have broken through the basement membrane at any point.

SPUTUM REPORTS.

There is nothing more disgusting to us than some sputum reports. Not a few of these come from laboratories of men who really know better, but who perhaps become careless. We have witnessed reports with the terse, "negative", stamped, written or typed followed merely by the signature of the examiner. It is perhaps not for us to say whether the worker shall or not report upon elastic tissue, cells, albumin and so on; it may be his privilege to regard a complete sputum examination as a search for the bacillus of Koch. But we do object to the meaningless "negative" passing as the last word from the medical laboratory—the sick man deserves a better fate. We make it a rule when reporting tubercle bacilli as absent, to give the physician some idea as to what kind of a search we carried out (and there are various methods carried out). Thus, for example, we state, "absent in three smears examined closely," or other words which will mean something to him.

But we have contended and still contend that a sputum examination means something more than to peep through a lens for red rods. The time has come when a sputum examination, done properly, is much more tedious (and fascinating) than a urinalysis, and when properly completed and reported, the fee should be twice or thrice that charged for the urinalysis.

OTHER REPORTS.

In general the same rules as given above apply to blood, gastric, pus and other reports, and it is not necessary to consider these in detail. If medical diagnosis reaches perfection anywhere, it is in the medical laboratory. Here are found the men who dare not guess, but who must work out problems with an accuracy demanded from no other class of specialists. Their records are permanent. These reports may travel across the continent and reach the hands of the greatest clinicians of the world. The future sentence upon the medical laboratory will be passed by the men who are reading these reports, and by these we will be judged.

This plea is to the laboratory man. Prepare well your exhibit for the eyes of the jurors.

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EDITORIALS

The Diagnosis and Treatment of Paresis

THREE papers lately published pertain to the very interesting subject of the serological diagnosis of general paresis and its treatment by the newer topical methods.

Lowery[1] publishes the results of four years' experience at Danvers with the serum and spinal fluid Wassermann. In 1,714 admissions since May, 1912, serum Wassermann has been done on 1,600 and spinal fluid Wassermann on 276. The cases were unselected. Two human heart, cholesterin fortified, and one luetic fetal liver extract were used as antigens in each test. Of the sera 16 per cent were positive, 3.87 per cent were doubtful and 80.13 per cent were negative. Of cases not showing a positive serum reaction (i.e., of 5 doubtful and 12 negative sera) 17 gave a positive spinal fluid Wassermann. In this series therefore one per cent of positive diagnosis was gained by performance of lumbar puncture.

It is of interest that 7.6 per cent of cases not clinically paresis gave positive serological results. Of the cases clinically paretic 87.5 per cent were positive, 10.3 negative and 2.2 doubtful. Other psychoses present some interesting findings: 10 per cent of imbeciles were syphilitic and in a group diagnosed

"pre-senile delusional insanity," corresponding perhaps to the familiar "involutional depression" there were 16 per cent of positive findings. It is also worth noting that in less than half of the positive cases was a positive history obtainable. However, mental incapacity would explain this to a great extent.

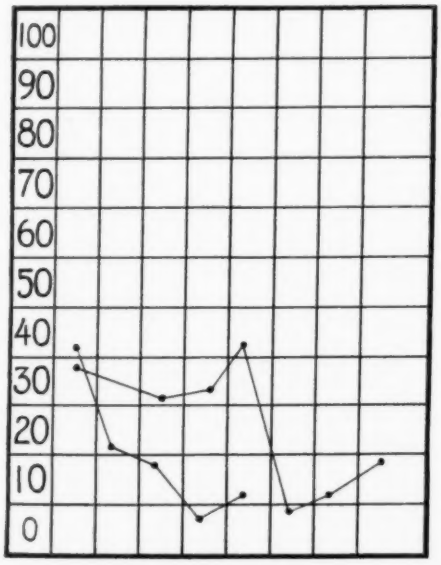
Evans and Thorne[2] present conclusions of the value of salvarsanized serum in the treatment of 15 cases of paresis; one of which was treated both intraspinally and intracranially. The number of intraspinous injections average about six for each patient. They summarized their results; three patients showed mental and physical improvement, but of these only one has remained well; one has withdrawn from observation and one died of convulsions less than ten months after the conclusion of the treatment; five showed physical improvement only and these two died about a year after discontinuance; three showed no improvement at all. They believe that improvement is no greater than with older methods and point out the fact that mere lumbar puncture will bring down the cell count. However, some objections could be raised to their work and conclusions. They have used the Swift-Ellis technic exclusively. By this method an average of 0.16 mg. of Salvarsan per c.c. of serum is obtained, though variations are enormous and range from 0.004 to 0.05. In Ogilvie's technic from 0.3 to 0.5 mg. are added directly to the serum *in vitro* so that the dose becomes definite and this improvement has been recognized by Swift in his last publication.[3] Besides, Evans and Thorne did not resume treatment when relapses appeared. In summarizing they say: "If the treatments are given at shorter intervals one is merely withdrawing part of the serum last injected as it takes nearly two weeks for the serum to be absorbed from the sub-arachnoid space." Though moderately familiar with the literature of intraspinous medication the present writer has never seen any data on the time of absorption and no authority is quoted for the statement referred to. Their conclusions that the intracranial route is unnecessary is based on one case only and is therefore negligible.

Wardner[4] reviews this method from safer ground, advocating it on the basis of fourteen cases treated by the intracranial method continuing a report on six cases of a year ago. Whenever possible relapses have been met with renewal of treatment and the treatments have totalled 82. His results are quoted verbatim: "To sum up of the fourteen cases five improved sufficiently to go back to their work and, to date, have remained well for from seven to eleven months. At the end of eleven months one of these cases had a bad relapse. He was immediately brought back to the hospital and he responded immediately to additional application of the serum. At present he is well mentally and physically and has parole of the grounds. Three other well developed cases have improved sufficiently to be put on parole of the grounds and are doing efficient work about the hospital. Three others have shown fairly marked physical and mental improvement, but cannot yet be trusted at large. Two have died." It is worth noting that neither died directly of cerebral syphilis, one had bronchopneumonia and one a cerebral cyst certified postmortem and apparently of earlier origin than the treatments.

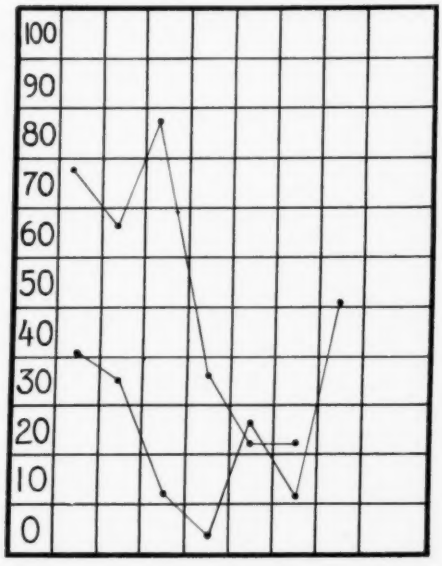
Such poor results as are reported by Evans and Thorne are fortunately not common. It is gratifying, however, that they have recognized the practical importance of the experimental work of Cushing, Weed and Wegefarth.[5] In a previous note[6] the present writer called attention to the fact that in the contro-

versy over the value of the various topical methods of treating cerebrospinal syphilis few seem to have realized the point of this research which demonstrated that, with pressures only very slightly and surely not dangerously above normal, the cerebral subarachnoid and perivascular and perineuronal spaces could be injected from the spinal canal. However, to reject the intracranial route on the basis of this and a single personal experience is not justified in the face of the excellent results reported especially by Wardner.

The value of laboratory findings comes in question again. Evans and Thorne contend that the fall in the cell count only parallels that produced by simple lumbar puncture and present "curves" comparing four treated with four untreated cases. Their claim that the curves "correspond well" is scarcely tenable as can readily be shown for their first two comparisons by the simple de-



No. 1



No. 2.

vice of superimposing them as in Figs. 1 and 2. Besides in case 1 while in the chart of the treated case the intervals on the abcissa represent a definite period (14 days), in that of the untreated case this unit is made to represent intervals varying as much as eleven and twenty-one days. If the time factor is immaterial the "curves" represent nothing—if material they are highly inaccurate. Too many and too uncertain factors enter into the cell count to plot it as a simple function of the time. Engineers and mathematicians would smile at a curve such as that of untreated case 4 plotted from four observations! At best these charts are good graphic diagrams but to attempt to compare them as curves is poor medicine and poor mathematics.

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The Formation of Calculi

PATHOLOGIC calcification occurs most commonly in dead tissue—in areas which have undergone necrosis. It also occurs less frequently in apparently healthy tissue. When the latter happens there is usually associated with it some severe disease of bone. Between calcification as we usually mean it, and concrement formation there is no discoverable fundamental difference. The superficial difference is that concrement formation takes place in a fluid medium, i.e., a more dilute colloidal solution than the one represented by that which forms the basis of areas of necrosis or of normal tissue. In ossification (i.e., normal calcification) the fundamental process is again the same, the only difference being that after calcification the process of organization proceeds in an orderly fashion to produce normal bone. Ossification occurs also under pathologic conditions, and produces true bone which however tends to have a more or less disorderly arrangement. Abnormal ossification occurs in such diseases as rickets and congenital syphilis.

In all these cases the matrix (whether fluid or solid) in which calcification is to take place, is saturated with lime salts, held in solution by virtue of the colloidal condition of the matrix, and so held because of the fact that these salts, chiefly carbonates, and phosphates of calcium (and urea) are more soluble in colloids (albumen) than in water. This may be illustrated by taking a solution of the normal body salts in serum and diluting it with water. The carbonates and phosphates of calcium are precipitated. Variations in alkalinity produce similar results. For instance, if one takes

KCl	0.40
$CaCl_2 + 6\ H_2O$	0.62
$MgCl_2 + 6\ H_2O$	0.37
NaCl	5.90
$NaH_2PO_4 + 1\ H_2O$	0.236
$NaHCO_3$	3.53
H_2O	1000.0

one finds that very soon a precipitation of calcium carbonate and phosphate appears.[1] If, however, serum colloids are added to the solution, no precipitation occurs.

Also Pauli and Samec have shown that, especially in the case of the difficultly soluble electrolytes, the presence of albumen makes a very considerable difference in the salt content of the fluid, as follows:

	In 100 grams H_2O.	In 100 grams serum.
Calcium sulphate	0.223	0.226
Calcium phosphate	0.011	0.021
Calcium carbonate	0.004	0.023
Silicic acid	0.023	0.030
Uric acid	0.040	0.057

Bechhold and Ziegler[2] showed that an electrolyte from serum albumen solution with an albumen content equal to that of defibrinated serum (7.6 per cent) uric acid was much more soluble than in water, while the reverse was true of sodium urate.

	In 100 grams serum albumen.	In H_2O.
Uric acid	0.02	0.00645
Monosodium urate	0.055	0.12—0.15

They also remarked that the slightest traces of sodium bicarbonate (in the case of uric acid and of sodium urate) influenced the solubilities.

From the fact that hydrogen and hydroxyl-ions have a remarkable influence upon the water content of albumen, it will be realized that these substances will also have a marked influence upon the amounts of salts held in solution in such fluids as serum and protoplasm.

What happens in ossification or calcification may be that these tissues, living or dead, and saturated to a greater or less degree with serum and salts, are changed sufficiently in reaction, or in water content, to bring about a deposition of the more difficultly soluble salts. Such a process may be facilitated or prevented by certain products of protein metabolism. It has been shown, for example, that the albumoses act almost exclusively upon the calcium carbonate to keep it in solution, while at the same time the phosphate becomes less soluble.[3]

Were the composition of the fluids of the body the same, undoubtedly we should have but one type of salt precipitation to deal with in studying calcification and concrement formation. As it is, the fluids vary, the common type is that of which we have been speaking, but under certain conditions of metabolism the urates of the body fluids are increased in concentration and then, instead of calcium salts, the deposits are composed of urates, which, like the calcium salts, are more readily soluble in serum than in water. In gout deposits urates are distributed in various parts of the body, and in a condition called "calcium gout," described by Schmidt, deposits of calcium produce an analogous picture. In the fluids of the body, as in the tissues, the type of deposit depends upon the composition of the fluid. In bile, cholesterol and bile salt calculi occur; in the urine, uric acid and urate "stones."

All concrements are formed about a nucleus and are held together by some organic material which acts as a "binding substance." Bacteria, epithelial cells, mucin, a foreign body, coagulated protein, all may act as nuclei about which precipitation occurs, and about it the particles are held together by mucin or fibrin, or perhaps other substances. In the case of urine, it must be remembered that very little colloidal material is present, so little that oftentimes not sufficient exists to bind together precipitates. When this is true urinary sand is formed instead of calculi. Moreover what binding substance is present is apt to be of a reversible nature, and therefore is inefficient. In the presence of inflammation,

however, fibrin, which is irreversible, is produced and this forms an excellent material for binding precipitates. In this connection it is interesting that H. Schade in his experiments with urinary calculus formation, found that fibrin was absolutely necessary.[4]

In the case of gall stones, apparently the only necessary things to assist calculus formation, are fats or fatty acids, for a few drops only of an oil or fat is all that is necessary to produce a precipitate of cholesterin from a solution. The precipitate is first an amorphous mass similar to that which Naunyn believed to be the starting point of calculi. Following this effect radial crystallization occurs about the amorphous calculi. The necessary fats can be produced by splitting of the fatty acid salts and cholates which are present in bile, and this splitting can be accomplished by *B. coli, B. typhosus, B. pyocyaneus* or *B. proteus.* Staphylococcus does not do this. If it be said that oversaturation of the bile is necessary before precipitation occurs, it may also be said that any reduction of the normal alkalinity of bile produces such an effect, and that growth of various organisms may accomplish such a result. The most important bacterial causes of gall stones are bacteria of the colon-typhoid group which act in reducing alkalinity, splitting the cholates and soaps of the bile, and adding some fibrin to it. Accordingly they satisfy all the needs of the situation in the production of cholesterin stones. But many gall stones contain also bilirubin and calcium in large amounts, a combination which does not occur normally in the bile. In weak alkaline solutions, calcium combines with bilirubin, especially in the presence of certain albumens. In catarrhs of the gall bladder in which the mucus content is increased, the alkalinity is increased and accordingly the conditions are satisfactory for the production of bilirubin—calcium calculi. In the case of calculi of mixed structure, variations first in the direction of alkalinity and then toward acidity give the necessary conditions.

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